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(54) Title: NEISSERIA MENINGITIDIS POLYPEPTIDES

(57) Abstract: The invention provides BASB047, BASB054, BASB068 and BASB069 polypeptides, and polynucleotides encoding BASB047, BASB054, BASB068 and BASB069 polypeptides and methods for producing such polypeptides by recombinant techniques. Also provided are diagnostic, prophylactic and therapeutic uses.

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## Novel Compounds

### FIELD OF THE INVENTION

This invention relates to polynucleotides, (herein referred to as "BASB047  
5 polynucleotide(s)", "BASB054 polynucleotide(s)", "BASB068 polynucleotide(s)" and  
"BASB069 polynucleotide(s)"), polypeptides encoded by them (referred to herein as  
"BASB047", "BASB054", "BASB068" and "BASB069" respectively or "BASB047  
polypeptide(s)", "BASB054 polypeptide(s)", "BASB068 polypeptide(s)" and "BASB069  
polypeptide(s)" respectively), recombinant materials and methods for their production. In  
10 another aspect, the invention relates to methods for using such polypeptides and  
polynucleotides, including vaccines against bacterial infections. In a further aspect, the  
invention relates to diagnostic assays for detecting infection of certain pathogens.

### BACKGROUND OF THE INVENTION

15 *Neisseria meningitidis* (meningococcus) is a Gram-negative bacterium frequently isolated  
from the human upper respiratory tract. It occasionally causes invasive bacterial diseases  
such as bacteremia and meningitis. The incidence of meningococcal disease shows  
geographical seasonal and annual differences (Schwartz, B., Moore, P.S., Broome, C.V.;  
Clin. Microbiol. Rev. 2 (Supplement), S18-S24, 1989). Most disease in temperate countries  
20 is due to strains of serogroup B and varies in incidence from 1-10/100,000/year total  
population sometimes reaching higher values (Kaczmarski, E.B. (1997), Commun. Dis.  
Rep. Rev. 7: R55-9, 1995; Scholten, R.J.P.M., Bijlmer, H.A., Poolman, J.T. et al. Clin.  
Infect. Dis. 16: 237-246, 1993; Cruz, C., Pavez, G., Aguilar, E., et al. Epidemiol. Infect.  
105: 119-126, 1990).

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Epidemics dominated by serogroup A meningococci, mostly in central Africa, are  
encountered, sometimes reaching levels up to 1000/100,000/year (Schwartz, B., Moore,  
P.S., Broome, C.V. Clin. Microbiol. Rev. 2 (Supplement), S18-S24, 1989). Nearly all cases  
as a whole of meningococcal disease are caused by serogroup A, B, C, W-135 and Y

meningococci and a tetravalent A, C, W-135, Y polysaccharide vaccine is available (Armand, J., Arminjon, F., Mynard, M.C., Lafaix, C., J. Biol. Stand. 10: 335-339, 1982).

5 The polysaccharide vaccines are currently being improved by way of chemical conjugating them to carrier proteins (Lieberman, J.M., Chiu, S.S., Wong, V.K., et al. JAMA 275 : 1499-1503, 1996).

10 A serogroup B vaccine is not available, since the B capsular polysaccharide was found to be nonimmunogenic, most likely because it shares structural similarity to host components (Wyle, F.A., Artenstein, M.S., Brandt, M.L. et al. J. Infect. Dis. 126: 514-522, 1972; Finne, J.M., Leinonen, M., Mäkelä, P.M. Lancet ii.: 355-357, 1983).

15 For many years efforts have been initiated and carried out to develop meningococcal outer membrane based vaccines (de Moraes, J.C., Perkins, B., Camargo, M.C. et al. Lancet 340: 1074-1078, 1992; Bjune, G., Hoiby, E.A. Gronnesby, J.K. et al. 338: 1093-1096, 1991). Such vaccines have demonstrated efficacies from 57% - 85% in older children (>4 years) and adolescents.

20 Many bacterial outer membrane components are present in these vaccines, such as PorA, PorB, Rmp, Opc, Opa, FrpB and the contribution of these components to the observed protection still needs further definition. Other bacterial outer membrane components have been defined by using animal or human antibodies to be potentially relevant to the induction of protective immunity, such as TbpB and NspA (Martin, D., Cadieux, N., Hamel, J., Brodeux, B.R., J. Exp. Med. 185: 1173-1183, 1997; Lissolo, L., Maître-Wilmotte, C., 25 Dumas, p. et al., Inf. Immun. 63: 884-890, 1995). The mechanisms of protective immunity will involve antibody mediated bactericidal activity and opsonophagocytosis.

A bacteremia animal model has been used to combine all antibody mediated mechanisms (Saukkonen, K., Leinonen, M., Abdillahi, H. Poolman, J. T. Vaccine 7: 325-328, 1989). It is

generally accepted that the late complement component mediated bactericidal mechanism is crucial for immunity against meningococcal disease (Ross, S.C., Rosenthal P.J., Berberic, H.M., Densen, P. J. Infect. Dis. 155: 1266-1275, 1987).

- 5 The frequency of *Neisseria meningitidis* infections has risen dramatically in the past few decades. This has been attributed to the emergence of multiply antibiotic resistant strains and an increasing population of people with weakened immune systems. It is no longer uncommon to isolate *Neisseria meningitidis* strains that are resistant to some or all of the standard antibiotics. This phenomenon has created an unmet medical need and demand for  
10 new anti-microbial agents, vaccines, drug screening methods, and diagnostic tests for this organism.

#### SUMMARY OF THE INVENTION

- 15 The present invention relates to BASB047, BASB054, BASB068 and BASB069, in particular BASB047, BASB054, BASB068 and BASB069 polypeptides and BASB047, BASB054, BASB068 and BASB069 polynucleotides, recombinant materials and methods for their production. In another aspect, the invention relates to methods for using such polypeptides and polynucleotides, including prevention and treatment of microbial  
20 diseases, amongst others. In a further aspect, the invention relates to diagnostic assays for detecting diseases associated with microbial infections and conditions associated with such infections, such as assays for detecting expression or activity of BASB047, BASB054, BASB068 and BASB069 polynucleotides or polypeptides.
- 25 Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following descriptions and from reading the other parts of the present disclosure.

## DESCRIPTION OF THE INVENTION

The invention relates to BASB047, BASB054, BASB068 and BASB069 polypeptides and polynucleotides as described in greater detail below. The invention relates especially to BASB047, BASB054, BASB068 and BASB069 having the nucleotide and amino acid sequences set out in SEQ ID NO:1,3,5,7 and SEQ ID NO:2,4,6,8 respectively. It is understood that sequences recited in the Sequence Listing below as "DNA" represent an exemplification of one embodiment of the invention, since those of ordinary skill will recognize that such sequences can be usefully employed in polynucleotides in general, including ribopolynucleotides.

### Polypeptides

In one aspect of the invention there are provided polypeptides of *Neisseria meningitidis* referred to herein as "BASB047", "BASB054", "BASB068" and "BASB069", and "BASB047 polypeptides", "BASB054 polypeptides", "BASB068 polypeptides" and "BASB069 polypeptides" as well as biologically, diagnostically, prophylactically, clinically or therapeutically useful variants thereof, and compositions comprising the same.

The present invention further provides for:

- (a) an isolated polypeptide which comprises an amino acid sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:2.
- (b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:1 over the entire length of SEQ ID NO:1.
- (c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:2.

The BASB043 polypeptide provided in SEQ ID NO:2 is the BASB047 polypeptide from *Neisseria meningitidis* strain ATCC13090.

- 5 The invention also provides an immunogenic fragment of a BASB047 polypeptide, that is, a contiguous portion of the BASB047 polypeptide which has the same or substantially the same immunogenic activity as the polypeptide comprising the amino acid sequence of SEQ ID NO:2. That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the BASB047 polypeptide.
- 10 Such an immunogenic fragment may include, for example, the BASB047 polypeptide lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of BASB047 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet
- 15 more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:2 over the entire length of SEQ ID NO:2.

The present invention further provides for:

- (a) an isolated polypeptide which comprises an amino acid sequence which has at least
- 20 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:4.
- (b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to
- 25 SEQ ID NO:3 over the entire length of SEQ ID NO:3.
- (c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:4

The BASB054 polypeptide provided in SEQ ID NO:4 is the BASB054 polypeptide from *Neisseria meningitidis* strain ATCC13090.

- 5 The invention also provides an immunogenic fragment of a BASB054 polypeptide, that is, a contiguous portion of the BASB054 polypeptide which has the same or substantially the same immunogenic activity as the polypeptide comprising the amino acid sequence of SEQ ID NO:4. That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the BASB054 polypeptide.
- 10 Such an immunogenic fragment may include, for example, the BASB054 polypeptide lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of BASB054 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet
- 15 more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:4 over the entire length of SEQ ID NO:4.

The present invention further provides for:

- (a) an isolated polypeptide which comprises an amino acid sequence which has at least
- 20 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:6.
- (b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to
- 25 SEQ ID NO:5 over the entire length of SEQ ID NO:5.
- (c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:6



The BASB068 polypeptide provided in SEQ ID NO:6 is the BASB068 polypeptide from *Neisseria meningitidis* strain ATCC13090.

- 5 The invention also provides an immunogenic fragment of a BASB068 polypeptide, that is, a contiguous portion of the BASB068 polypeptide which has the same or substantially the same immunogenic activity as the polypeptide comprising the amino acid sequence of SEQ ID NO:6. That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the BASB068 polypeptide.
- 10 Such an immunogenic fragment may include, for example, the BASB068 polypeptide lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of BASB068 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet
- 15 more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:6 over the entire length of SEQ ID NO:6.

The present invention further provides for:

- (a) an isolated polypeptide which comprises an amino acid sequence which has at least
- 20 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:8.
- (b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to
- 25 SEQ ID NO:7 over the entire length of SEQ ID NO:7.
- (c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:8

The BASB069 polypeptide provided in SEQ ID NO:8 is the BASB069 polypeptide from *Neisseria meningitidis* strain ATCC13090.

- 5 The invention also provides an immunogenic fragment of a BASB069 polypeptide, that is, a contiguous portion of the BASB069 polypeptide which has the same or substantially the same immunogenic activity as the polypeptide comprising the amino acid sequence of SEQ ID NO:8. That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the BASB069 polypeptide.
- 10 Such an immunogenic fragment may include, for example, the BASB069 polypeptide lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of BASB069 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet
- 15 more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:8 over the entire length of SEQ ID NO:8.

- A fragment is a polypeptide having an amino acid sequence that is entirely the same as part
- 20 but not all of any amino acid sequence of any polypeptide of the invention. As with BASB047, BASB054, BASB068 and BASB069 polypeptides, fragments may be "free-standing", or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region in a single larger polypeptide.

- 25 Preferred fragments include, for example, truncation polypeptides having a portion of an amino acid sequence of SEQ ID NO:2,4,6,8 or of variants thereof, such as a continuous series of residues that includes an amino- and/or carboxyl-terminal amino acid sequence. Degradation forms of the polypeptides of the invention produced by or in a host cell, are also preferred. Further preferred are fragments characterized by structural or functional

attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions.

Further preferred fragments include an isolated polypeptide comprising an amino acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous amino acids from the amino acid sequence of SEQ ID NO:2,4,6,8 or an isolated polypeptide comprising an amino acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous amino acids truncated or deleted from the amino acid sequence of SEQ ID NO:2,4,6,8.

Fragments of the polypeptides of the invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, these fragments may be employed as intermediates for producing the full-length polypeptides of the invention.

Particularly preferred are variants in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acids are substituted, deleted, or added in any combination.

The polypeptides, or immunogenic fragments, of the invention may be in the form of the "mature" protein or may be a part of a larger protein such as a precursor or a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production. Furthermore, addition of exogenous polypeptide or lipid tail or polynucleotide sequences to increase the immunogenic potential of the final molecule is also considered.

In one aspect, the invention relates to genetically engineered soluble fusion proteins comprising a polypeptide of the present invention, or a fragment thereof, and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa.

Furthermore, this invention relates to processes for the preparation of these fusion proteins by genetic engineering, and to the use thereof for drug screening, diagnosis and therapy. A further aspect of the invention also relates to polynucleotides encoding such fusion proteins. Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

The proteins may be chemically conjugated, or expressed as recombinant fusion proteins allowing increased levels to be produced in an expression system as compared to non-fused protein. The fusion partner may assist in providing T helper epitopes (immunological fusion partner), preferably T helper epitopes recognised by humans, or assist in expressing the protein (expression enhancer) at higher yields than the native recombinant protein. Preferably the fusion partner will be both an immunological fusion partner and expression enhancing partner.

Fusion partners include protein D from *Haemophilus influenzae* and the non-structural protein from influenza virus, NS1 (hemagglutinin). Another fusion partner is the protein known as LytA. Preferably the C terminal portion of the molecule is used. LytA is derived from *Streptococcus pneumoniae* which synthesize an N-acetyl-L-alanine amidase, amidase LytA, (coded by the lytA gene {Gene, 43 (1986) page 265-272}) an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LytA protein is responsible for the affinity to

the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E.coli* C-LytA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LytA fragment at its amino terminus has been described {Biotechnology: 10, (1992) page 795-798}. It is possible to use the repeat portion of the LytA molecule found in the C terminal end starting at residue 178, for example residues 188 - 305.

The present invention also includes variants of the aforementioned polypeptides, that is polypeptides that vary from the referents by conservative amino acid substitutions, whereby a residue is substituted by another with like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr.

Polypeptides of the present invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

It is most preferred that a polypeptide of the invention is derived from *Neisseria meningitidis*, however, it may preferably be obtained from other organisms of the same taxonomic genus. A polypeptide of the invention may also be obtained, for example, from organisms of the same taxonomic family or order.

### Polynucleotides

It is an object of the invention to provide polynucleotides that encode BASB047 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB047.

In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB047 polypeptides comprising a sequence set out in SEQ ID NO:1 which includes a full length gene, or a variant thereof.

5

The BASB047 polynucleotide provided in SEQ ID NO:1 is the BASB047 polynucleotide from *Neisseria meningitidis* strains ATCC13090.

10 As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB047 polypeptides and polynucleotides, particularly *Neisseria meningitidis* BASB047 polypeptides and polynucleotides, including, for example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and Z-DNAs. Further embodiments of the invention include biologically,  
15 diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

Another aspect of the invention relates to isolated polynucleotides, including at least one full length gene, that encodes a BASB047 polypeptide having a deduced amino acid sequence of SEQ ID NO:2 and polynucleotides closely related thereto and variants thereof.

20

In another particularly preferred embodiment of the invention there is a BASB047 polypeptide from *Neisseria meningitidis* comprising or consisting of an amino acid sequence of SEQ ID NO:2 or a variant thereof.

25 Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:1 a polynucleotide of the invention encoding BASB047 polypeptide may be obtained using standard cloning and screening methods, such as those for cloning and sequencing chromosomal DNA fragments from bacteria using *Neisseria meningitidis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide

sequence of the invention, such as a polynucleotide sequence given in SEQ ID NO:1 typically a library of clones of chromosomal DNA of *Neisseria meningitidis* in *E.coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from the original polypeptide or polynucleotide sequence it is then possible to extend the polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:1 was discovered in a DNA library derived from *Neisseria meningitidis*.

Moreover, the DNA sequence set out in SEQ ID NO:1 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:2 with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those skilled in the art.

The polynucleotide of SEQ ID NO:1, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 1201 of SEQ ID NO:1, encodes the polypeptide of SEQ ID NO:2.

In a further aspect, the present invention provides for an isolated polynucleotide comprising or consisting of:

- (a) a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:1 over the entire length of SEQ ID NO:1; or
- 5 (b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:2 over the entire length of SEQ ID NO:2.

10 A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO:1 or a fragment thereof; and isolating a full-length gene and/or

15 genomic clones containing said polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO:1. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding

20 sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals

25 (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals. The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker



sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc. Natl. Acad. Sci., USA* 86: 821-824 (1989), or an HA peptide tag (Wilson *et al.*, *Cell* 37: 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

The nucleotide sequence encoding BASB047 polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in nucleotides 1 to 1200 of SEQ ID NO:1. Alternatively it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2. The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Neisseria meningitidis* BASB047 having an amino acid sequence set out in SEQ ID NO:2. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:2. Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding BASB047 variants, that have the amino acid sequence of BASB047 polypeptide of SEQ ID NO:2 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted.

modified, deleted and/or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, that do not alter the properties and activities of BASB047 polypeptide.

- 5 Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB047 polypeptide having an amino acid sequence set out in SEQ ID NO:2 and polynucleotides that are complementary to such polynucleotides. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred.
- 10 Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.
- 15 Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of SEQ ID NO:1.

- In accordance with certain preferred embodiments of this invention there are provided
- 20 polynucleotides that hybridize, particularly under stringent conditions, to BASB047 polynucleotide sequences, such as those polynucleotides in SEQ ID NO:1.

- The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to
- 25 polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x

SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the polynucleotide sequences provided by the invention.

The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:1 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:1 or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding BASB047 and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the BASB047 gene. Such probes generally will comprise at least 15 nucleotide residues or base pairs. Preferably, such probes will have at least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

A coding region of a BASB047 gene may be isolated by screening using a DNA sequence provided in SEQ ID NO:1 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then

used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

It is an object of the invention to provide polynucleotides that encode BASB054 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB054.

- 5 In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB054 polypeptides comprising a sequence set out in SEQ ID NO:3 which includes a full length gene, or a variant thereof.

- The BASB054 polynucleotide provided in SEQ ID NO:3 is the BASB054  
10 polynucleotide from *Neisseria meningitidis* strains ATCC13090.

- As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB054 polypeptides and polynucleotides, particularly *Neisseria meningitidis* BASB054 polypeptides and polynucleotides, including, for  
15 example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

- 20 Another aspect of the invention relates to isolated polynucleotides, including at least one full length gene, that encodes a BASB054 polypeptide having a deduced amino acid sequence of SEQ ID NO:4 and polynucleotides closely related thereto and variants thereof.

- In another particularly preferred embodiment of the invention there is a BASB054  
25 polypeptide from *Neisseria meningitidis* comprising or consisting of an amino acid sequence of SEQ ID NO:4 or a variant thereof.

Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:3 a polynucleotide of the invention encoding BASB054 polypeptide may be obtained

using standard cloning and screening methods, such as those for cloning and sequencing chromosomal DNA fragments from bacteria using *Neisseria meningitidis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in SEQ ID NO:3

5 typically a library of clones of chromosomal DNA of *Neisseria meningitidis* in *E.coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers

10 designed from the original polypeptide or polynucleotide sequence it is then possible to extend the polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A*

15 *LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:3 was discovered in a DNA

20 library derived from *Neisseria meningitidis*.

Moreover, the DNA sequence set out in SEQ ID NO:3 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:4 with a deduced molecular weight that can be calculated using amino acid residue

25 molecular weight values well known to those skilled in the art.

The polynucleotide of SEQ ID NO:3, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 2407 of SEQ ID NO:3, encodes the polypeptide of SEQ ID NO:4.

In a further aspect, the present invention provides for an isolated polynucleotide comprising or consisting of:

- (a) a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:3 over the entire length of SEQ ID NO:3; or
- (b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:4 over the entire length of SEQ ID NO:4.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO:3 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO:3. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals.

The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc. Natl. Acad. Sci., USA* 86: 821-824 (1989), or an HA peptide tag (Wilson *et al.*, *Cell* 37: 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

The nucleotide sequence encoding BASB054 polypeptide of SEQ ID NO:4 may be identical to the polypeptide encoding sequence contained in nucleotides 1 to 2406 of SEQ ID NO:3. Alternatively it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:4.

The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Neisseria meningitidis* BASB054 having an amino acid sequence set out in SEQ ID NO:4. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:4. Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.



Further particularly preferred embodiments are polynucleotides encoding BASB054 variants, that have the amino acid sequence of BASB054 polypeptide of SEQ ID NO:4 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are  
5 silent substitutions, additions and deletions, that do not alter the properties and activities of BASB054 polypeptide.

Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB054 polypeptide having  
10 an amino acid sequence set out in SEQ ID NO:4 and polynucleotides that are complementary to such polynucleotides. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%,  
15 and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of  
20 SEQ ID NO:3.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to BASB054 polynucleotide sequences, such as those polynucleotides in SEQ ID NO:3.  
25

The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization

conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the polynucleotide sequences provided by the invention.

The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:3 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:3 or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding BASB054 and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the BASB054 gene. Such probes generally will comprise at least 15 nucleotide residues or base pairs. Preferably, such probes will have at least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

A coding region of a BASB054 gene may be isolated by screening using a DNA sequence provided in SEQ ID NO:3 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

5

It is an object of the invention to provide polynucleotides that encode BASB068 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB068.

- 5 In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB068 polypeptides comprising a sequence set out in SEQ ID NO:5 which includes a full length gene, or a variant thereof.

The BASB068 polynucleotide provided in SEQ ID NO:5 is the BASB068  
10 polynucleotide from *Neisseria meningitidis* strains ATCC13090.

As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB068 polypeptides and polynucleotides, particularly *Neisseria meningitidis* BASB068 polypeptides and polynucleotides, including, for  
15 example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

- 20 Another aspect of the invention relates to isolated polynucleotides, including at least one full length gene, that encodes a BASB068 polypeptide having a deduced amino acid sequence of SEQ ID NO:6 and polynucleotides closely related thereto and variants thereof.

In another particularly preferred embodiment of the invention there is a BASB068  
25 polypeptide from *Neisseria meningitidis* comprising or consisting of an amino acid sequence of SEQ ID NO:6 or a variant thereof.

Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:5 a polynucleotide of the invention encoding BASB068 polypeptide may be obtained

using standard cloning and screening methods, such as those for cloning and sequencing chromosomal DNA fragments from bacteria using *Neisseria meningitidis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in SEQ ID NO:5

5 typically a library of clones of chromosomal DNA of *Neisseria meningitidis* in *E.coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers

10 designed from the original polypeptide or polynucleotide sequence it is then possible to extend the polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A*

15 *LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:5 was discovered in a DNA

20 library derived from *Neisseria meningitidis*.

Moreover, the DNA sequence set out in SEQ ID NO:5 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:6 with a deduced molecular weight that can be calculated using amino acid residue  
25 molecular weight values well known to those skilled in the art.

The polynucleotide of SEQ ID NO:5, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 2014 of SEQ ID NO:5, encodes the polypeptide of SEQ ID NO:6.

In a further aspect, the present invention provides for an isolated polynucleotide comprising or consisting of:

- 5 (a) a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:5 over the entire length of SEQ ID NO:5; or
- (b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID  
10 NO:6 over the entire length of SEQ ID NO:6.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization  
15 conditions (for example, using a temperature in the range of 45 – 65°C and an SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO:5 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

20 The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO:5. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or  
25 prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals.

The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc. Natl. Acad. Sci., USA* 86: 821-824 (1989), or an HA peptide tag (Wilson *et al.*, *Cell* 37: 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

10

The nucleotide sequence encoding BASB068 polypeptide of SEQ ID NO:6 may be identical to the polypeptide encoding sequence contained in nucleotides 1 to 2013 of SEQ ID NO:5. Alternatively it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:6.

15 The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Neisseria meningitidis* BASB068 having an amino acid sequence set out in SEQ ID NO:6. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

20

25 The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:6. Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding BASB068 variants, that have the amino acid sequence of BASB068 polypeptide of SEQ ID NO:6 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are  
5 silent substitutions, additions and deletions, that do not alter the properties and activities of BASB068 polypeptide.

Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB068 polypeptide having  
10 an amino acid sequence set out in SEQ ID NO:6 and polynucleotides that are complementary to such polynucleotides. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%,  
15 and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of  
20 SEQ ID NO:5.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to BASB068 polynucleotide sequences, such as those polynucleotides in SEQ ID NO:5.  
25

The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization



conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the polynucleotide sequences provided by the invention.

The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:5 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:5 or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding BASB068 and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the BASB068 gene. Such probes generally will comprise at least 15 nucleotide residues or base pairs. Preferably, such probes will have at least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

A coding region of a BASB068 gene may be isolated by screening using a DNA sequence provided in SEQ ID NO:5 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

5

It is an object of the invention to provide polynucleotides that encode BASB069 polypeptides, particularly polynucleotides that encode the polypeptide herein designated BASB069.

- 5 In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding BASB069 polypeptides comprising a sequence set out in SEQ ID NO:7 which includes a full length gene, or a variant thereof.

- 10 The BASB069 polynucleotide provided in SEQ ID NO:7 is the BASB069 polynucleotide from *Neisseria meningitidis* strains ATCC13090.

- 15 As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing BASB069 polypeptides and polynucleotides, particularly *Neisseria meningitidis* BASB069 polypeptides and polynucleotides, including, for example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

- 20 Another aspect of the invention relates to isolated polynucleotides, including at least one full length gene, that encodes a BASB069 polypeptide having a deduced amino acid sequence of SEQ ID NO:8 and polynucleotides closely related thereto and variants thereof.

- 25 In another particularly preferred embodiment of the invention there is a BASB069 polypeptide from *Neisseria meningitidis* comprising or consisting of an amino acid sequence of SEQ ID NO:8 or a variant thereof.

Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:7 a polynucleotide of the invention encoding BASB069 polypeptide may be obtained

using standard cloning and screening methods, such as those for cloning and sequencing chromosomal DNA fragments from bacteria using *Neisseria meningitidis* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in SEQ ID NO:7 typically a library of clones of chromosomal DNA of *Neisseria meningitidis* in *E.coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from the original polypeptide or polynucleotide sequence it is then possible to extend the polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:7 was discovered in a DNA library derived from *Neisseria meningitidis*.

Moreover, the DNA sequence set out in SEQ ID NO:7 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:8 with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those skilled in the art.

The polynucleotide of SEQ ID NO:7, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 2074 of SEQ ID NO:7, encodes the polypeptide of SEQ ID NO:8.

In a further aspect, the present invention provides for an isolated polynucleotide comprising or consisting of:

- (a) a polynucleotide sequence which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:7 over the entire length of SEQ ID NO:7; or
- (b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, more preferably at least 90% identity, yet more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:8 over the entire length of SEQ ID NO:8.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Neisseria meningitidis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising the sequence of SEQ ID NO:7 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in SEQ ID NO:7. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals.

The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc. Natl. Acad. Sci., USA* 86: 821-824 (1989), or an HA peptide tag (Wilson *et al.*, *Cell* 37: 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

The nucleotide sequence encoding BASB069 polypeptide of SEQ ID NO:8 may be identical to the polypeptide encoding sequence contained in nucleotides 1 to 2073 of SEQ ID NO:7. Alternatively it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:8.

The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Neisseria meningitidis* BASB069 having an amino acid sequence set out in SEQ ID NO:8. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:8. Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding BASB069 variants, that have the amino acid sequence of BASB069 polypeptide of SEQ ID NO:8 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are  
5 silent substitutions, additions and deletions, that do not alter the properties and activities of BASB069 polypeptide.

Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB069 polypeptide having  
10 an amino acid sequence set out in SEQ ID NO:8 and polynucleotides that are complementary to such polynucleotides. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%,  
15 and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as the mature polypeptide encoded by a DNA of  
20 SEQ ID NO:7.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to BASB069 polynucleotide sequences, such as those polynucleotides in SEQ ID NO:7.  
25

The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization

conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the polynucleotide sequences provided by the invention.

The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:7 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:7 or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding BASB069 and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the BASB069 gene. Such probes generally will comprise at least 15 nucleotide residues or base pairs. Preferably, such probes will have at least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.



A coding region of a BASB069 gene may be isolated by screening using a DNA sequence provided in SEQ ID NO:7 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

There are several methods available and well known to those skilled in the art to obtain full-length DNAs, or extend short DNAs, for example those based on the method of Rapid Amplification of cDNA ends (RACE) (see, for example, Frohman, *et al.*, *PNAS USA* 85: 8998-9002, 1988). Recent modifications of the technique, exemplified by the Marathon™ technology (Clontech Laboratories Inc.) for example, have significantly simplified the search for longer cDNAs. In the Marathon™ technology, cDNAs have been prepared from mRNA extracted from a chosen tissue and an 'adaptor' sequence ligated onto each end. Nucleic acid amplification (PCR) is then carried out to amplify the "missing" 5' end of the DNA using a combination of gene specific and adaptor specific oligonucleotide primers. The PCR reaction is then repeated using "nested" primers, that is, primers designed to anneal within the amplified product (typically an adaptor specific primer that anneals further 3' in the adaptor sequence and a gene specific primer that anneals further 5' in the selected gene sequence). The products of this reaction can then be analyzed by DNA sequencing and a full-length DNA constructed either by joining the product directly to the existing DNA to give a complete sequence, or carrying out a separate full-length PCR using the new sequence information for the design of the 5' primer.

The polynucleotides and polypeptides of the invention may be employed, for example, as research reagents and materials for discovery of treatments of and diagnostics for diseases, particularly human diseases, as further discussed herein relating to polynucleotide assays.

The polynucleotides of the invention that are oligonucleotides derived from a sequence of SEQ ID NOS:1 – 8 may be used in the processes herein as described, but preferably for PCR, to determine whether or not the polynucleotides identified herein in whole or in part are transcribed in bacteria in infected tissue. It is recognized that such sequences will also have utility in diagnosis of the stage of infection and type of infection the pathogen has attained.

The invention also provides polynucleotides that encode a polypeptide that is the mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the mature polypeptide (when the mature form has more than one polypeptide chain, for instance). Such sequences may play a role in processing of a protein from precursor to a mature form, may allow protein transport, may lengthen or shorten protein half-life or may facilitate manipulation of a protein for assay or production, among other things. As generally is the case *in vivo*, the additional amino acids may be processed away from the mature protein by cellular enzymes.

For each and every polynucleotide of the invention there is provided a polynucleotide complementary to it. It is preferred that these complementary polynucleotides are fully complementary to each polynucleotide with which they are complementary.

A precursor protein, having a mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When prosequences are removed such inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called proproteins.

In addition to the standard A, G, C, T/U representations for nucleotides, the term "N" may also be used in describing certain polynucleotides of the invention. "N" means that any of the four DNA or RNA nucleotides may appear at such a designated position in the DNA or RNA sequence. except it is preferred that N is not a nucleic acid that when taken in

combination with adjacent nucleotide positions, when read in the correct reading frame, would have the effect of generating a premature termination codon in such reading frame.

5 In sum, a polynucleotide of the invention may encode a mature protein, a mature protein plus a leader sequence (which may be referred to as a preprotein), a precursor of a mature protein having one or more prosequences that are not the leader sequences of a preprotein, or a preproprotein, which is a precursor to a proprotein, having a leader sequence and one or more prosequences, which generally are removed during processing steps that produce active and mature forms of the polypeptide.

10

In accordance with an aspect of the invention, there is provided the use of a polynucleotide of the invention for therapeutic or prophylactic purposes, in particular genetic immunization.

15 The use of a polynucleotide of the invention in genetic immunization will preferably employ a suitable delivery method such as direct injection of plasmid DNA into muscles (Wolff *et al.*, *Hum Mol Genet* (1992) 1: 363, Manthorpe *et al.*, *Hum. Gene Ther.* (1983) 4: 419), delivery of DNA complexed with specific protein carriers (Wu *et al.*, *J Biol Chem.* (1989) 264: 16985), coprecipitation of DNA with calcium phosphate (Benvenisty & Reshef, *PNAS USA*, (1986) 83: 9551), encapsulation of DNA in various forms of liposomes (Kaneda *et al.*, *Science* (1989) 243: 375), particle bombardment (Tang *et al.*, *Nature* (1992) 356:152, Eisenbraun *et al.*, *DNA Cell Biol* (1993) 12: 791) and *in vivo* infection using cloned retroviral vectors (Seeger *et al.*, *PNAS USA* (1984) 81: 5849).

## 25 Vectors, Host Cells, Expression Systems

The invention also relates to vectors that comprise a polynucleotide or polynucleotides of the invention, host cells that are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques. Cell-free

translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the invention.

5 Recombinant polypeptides of the present invention may be prepared by processes well known in those skilled in the art from genetically engineered host cells comprising expression systems. Accordingly, in a further aspect, the present invention relates to expression systems that comprise a polynucleotide or polynucleotides of the present invention, to host cells which are genetically engineered with such expression systems, and to the production of polypeptides of the invention by recombinant techniques.

10 For recombinant production of the polypeptides of the invention, host cells can be genetically engineered to incorporate expression systems or portions thereof or polynucleotides of the invention. Introduction of a polynucleotide into the host cell can be effected by methods described in many standard laboratory manuals, such as Davis, *et al.*,  
15 *BASIC METHODS IN MOLECULAR BIOLOGY*, (1986) and Sambrook, *et al.*, *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), such as, calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic  
20 introduction and infection.

Representative examples of appropriate hosts include bacterial cells, such as cells of streptococci, staphylococci, enterococci, *E. coli*, streptomyces, cyanobacteria, *Bacillus subtilis*, *Moraxella catarrhalis*, *Haemophilus influenzae* and *Neisseria meningitidis*; fungal  
25 cells, such as cells of a yeast, *Kluveromyces*, *Saccharomyces*, a basidiomycete, *Candida albicans* and *Aspergillus*; insect cells such as cells of *Drosophila* S2 and *Spodoptera* Sf9; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293, CV-1 and Bowes melanoma cells; and plant cells, such as cells of a gymnosperm or angiosperm.

A great variety of expression systems can be used to produce the polypeptides of the invention. Such vectors include, among others, chromosomal-, episomal- and virus-derived vectors, for example, vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal  
5 elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses, picornaviruses, retroviruses, and alphaviruses and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression system constructs may contain control regions that regulate as well as engender  
10 expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides and/or to express a polypeptide in a host may be used for expression in this regard. The appropriate DNA sequence may be inserted into the expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL*, (*supra*).

15 In recombinant expression systems in eukaryotes, for secretion of a translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide. These signals may be endogenous to the polypeptide or they may be  
20 heterologous signals.

Polypeptides of the present invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose  
25 chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, ion metal affinity chromatography (IMAC) is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during intracellular synthesis, isolation and or purification.

The expression system may also be a recombinant live microorganism, such as a virus or bacterium. The gene of interest can be inserted into the genome of a live recombinant virus or bacterium. Inoculation and *in vivo* infection with this live vector will lead to *in vivo* expression of the antigen and induction of immune responses. Viruses and bacteria used for this purpose are for instance: poxviruses (e.g; vaccinia, fowlpox, canarypox), alphaviruses (Sindbis virus, Semliki Forest Virus, Venezuelan Equine Encephalitis Virus), adenoviruses, adeno-associated virus, picornaviruses (poliovirus, rhinovirus), herpesviruses (varicella zoster virus, etc), Listeria, Salmonella, Shigella, Neisseria, BCG. These viruses and bacteria can be virulent, or attenuated in various ways in order to obtain live vaccines. Such live vaccines also form part of the invention.

#### Diagnostic, Prognostic, Serotyping and Mutation Assays

- 15 This invention is also related to the use of BASB047, BASB054, BASB068 or BASB069 polynucleotides and polypeptides of the invention for use as diagnostic reagents. Detection of BASB047, BASB054, BASB068 or BASB069 polynucleotides and/or polypeptides in a eukaryote, particularly a mammal, and especially a human, will provide a diagnostic method for diagnosis of disease, staging of disease or response of an infectious organism to drugs.
- 20 Eukaryotes, particularly mammals, and especially humans, particularly those infected or suspected to be infected with an organism comprising the BASB047, BASB054, BASB068 or BASB069 gene or protein, may be detected at the nucleic acid or amino acid level by a variety of well known techniques as well as by methods provided herein.
- 25 Polypeptides and polynucleotides for prognosis, diagnosis or other analysis may be obtained from a putatively infected and/or infected individual's bodily materials. Polynucleotides from any of these sources, particularly DNA or RNA, may be used directly for detection or may be amplified enzymatically by using PCR or any other amplification technique prior to analysis. RNA, particularly mRNA, cDNA and genomic DNA may also be used in the

- same ways. Using amplification, characterization of the species and strain of infectious or resident organism present in an individual, may be made by an analysis of the genotype of a selected polynucleotide of the organism. Deletions and insertions can be detected by a change in size of the amplified product in comparison to a genotype of a reference sequence
- 5 selected from a related organism, preferably a different species of the same genus or a different strain of the same species. Point mutations can be identified by hybridizing amplified DNA to labeled BASB047, BASB054, BASB068 or BASB069 polynucleotide sequences. Perfectly or significantly matched sequences can be distinguished from imperfectly or more significantly mismatched duplexes by DNase or RNase digestion, for
- 10 DNA or RNA respectively, or by detecting differences in melting temperatures or renaturation kinetics. Polynucleotide sequence differences may also be detected by alterations in the electrophoretic mobility of polynucleotide fragments in gels as compared to a reference sequence. This may be carried out with or without denaturing agents. Polynucleotide differences may also be detected by direct DNA or RNA sequencing. See,
- 15 for example, Myers *et al.*, *Science*, 230: 1242 (1985). Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase, V1 and S1 protection assay or a chemical cleavage method. See, for example, Cotton *et al.*, *Proc. Natl. Acad. Sci., USA*, 85: 4397-4401 (1985).
- 20 In another embodiment, an array of oligonucleotides probes comprising a BASB047, BASB054, BASB068 or BASB069 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of, for example, genetic mutations, serotype, taxonomic classification or identification. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular
- 25 genetics including gene expression, genetic linkage, and genetic variability (see, for example, Chee *et al.*, *Science*, 274: 610 (1996)).

Thus in another aspect, the present invention relates to a diagnostic kit which comprises:

- (a) a polynucleotide of the present invention, preferably the nucleotide sequence of SEQ ID NO:1,3,5,7 or a fragment thereof ;
- (b) a nucleotide sequence complementary to that of (a);
- (c) a polypeptide of the present invention, preferably the polypeptide of SEQ ID NO:2,4,6,8 or a fragment thereof; or
- (d) an antibody to a polypeptide of the present invention, preferably to the polypeptide of SEQ ID NO:2,4,6,8.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component. Such a kit will be of use in diagnosing a disease or susceptibility to a disease, among others.

This invention also relates to the use of polynucleotides of the present invention as diagnostic reagents. Detection of a mutated form of a polynucleotide of the invention, preferably of SEQ ID NO:1,3,5 or 7 which is associated with a disease or pathogenicity will provide a diagnostic tool that can add to, or define, a diagnosis of a disease, a prognosis of a course of disease, a determination of a stage of disease, or a susceptibility to a disease, which results from under-expression, over-expression or altered expression of the polynucleotide. Organisms, particularly infectious organisms, carrying mutations in such polynucleotide may be detected at the polynucleotide level by a variety of techniques, such as those described elsewhere herein.

Cells from an organism carrying mutations or polymorphisms (allelic variations) in a polynucleotide and/or polypeptide of the invention may also be detected at the polynucleotide or polypeptide level by a variety of techniques, to allow for serotyping, for example. For example, RT-PCR can be used to detect mutations in the RNA. It is particularly preferred to use RT-PCR in conjunction with automated detection systems, such as, for example, GeneScan. RNA, cDNA or genomic DNA may also be used for the same purpose. PCR. As an example. PCR primers complementary to a polynucleotide encoding



BASB047, BASB054, BASB068 or BASB069 polypeptide can be used to identify and analyze mutations.

5 The invention further provides primers with 1, 2, 3 or 4 nucleotides removed from the 5' and/or the 3' end. These primers may be used for, among other things, amplifying BASB047, BASB054, BASB068 or BASB069 DNA and/or RNA isolated from a sample derived from an individual, such as a bodily material. The primers may be used to amplify a polynucleotide isolated from an infected individual, such that the polynucleotide may then be subject to various techniques for elucidation of the polynucleotide sequence. In this way,  
10 mutations in the polynucleotide sequence may be detected and used to diagnose and/or prognose the infection or its stage or course, or to serotype and/or classify the infectious agent.

The invention further provides a process for diagnosing disease, preferably bacterial  
15 infections, more preferably infections caused by *Neisseria meningitidis*, comprising determining from a sample derived from an individual, such as a bodily material, an increased level of expression of polynucleotide having a sequence of SEQ ID NO:1,3,5 or 7. Increased or decreased expression of a BASB047, BASB054, BASB068 or BASB069 polynucleotide can be measured using any one of the methods well known in the art for the  
20 quantitation of polynucleotides, such as, for example, amplification, PCR, RT-PCR, RNase protection, Northern blotting, spectrometry and other hybridization methods.

In addition, a diagnostic assay in accordance with the invention for detecting over-expression of BASB047, BASB054, BASB068 or BASB069 polypeptide compared to  
25 normal control tissue samples may be used to detect the presence of an infection, for example. Assay techniques that can be used to determine levels of a BASB047, BASB054, BASB068 or BASB069 polypeptide, in a sample derived from a host, such as a bodily material, are well-known to those of skill in the art. Such assay methods include

radioimmunoassays, competitive-binding assays, Western Blot analysis, antibody sandwich assays, antibody detection and ELISA assays.

5 The polynucleotides of the invention may be used as components of polynucleotide arrays, preferably high density arrays or grids. These high density arrays are particularly useful for diagnostic and prognostic purposes. For example, a set of spots each comprising a different gene, and further comprising a polynucleotide or polynucleotides of the invention, may be used for probing, such as using hybridization or nucleic acid amplification, using a probe obtained or derived from a bodily sample, 10 to determine the presence of a particular polynucleotide sequence or related sequence in an individual. Such a presence may indicate the presence of a pathogen, particularly *Neisseria meningitidis*, and may be useful in diagnosing and/or prognosing disease or a course of disease. A grid comprising a number of variants of the polynucleotide sequence of SEQ ID NO:1,3,5,7 are preferred. Also preferred is a grid comprising a 15 number of variants of a polynucleotide sequence encoding the polypeptide sequence of SEQ ID NO:2,4,6 or 8.

### Antibodies

20 The polypeptides and polynucleotides of the invention or variants thereof, or cells expressing the same can be used as immunogens to produce antibodies immunospecific for such polypeptides or polynucleotides respectively.

In certain preferred embodiments of the invention there are provided antibodies against 25 BASB047, BASB054, BASB068 or BASB069 polypeptides or polynucleotides.

Antibodies generated against the polypeptides or polynucleotides of the invention can be obtained by administering the polypeptides and/or polynucleotides of the invention, or epitope-bearing fragments of either or both, analogues of either or both, or cells expressing

either or both, to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique known in the art that provides antibodies produced by continuous cell line cultures can be used. Examples include various techniques, such as those in Kohler, G. and Milstein, C., *Nature* 256: 495-497 (1975);  
5 Kozbor *et al.*, *Immunology Today* 4: 72 (1983); Cole *et al.*, pg. 77-96 in *MONOCLONAL ANTIBODIES AND CANCER THERAPY*, Alan R. Liss, Inc. (1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can be adapted to produce single chain antibodies to polypeptides or polynucleotides of this  
10 invention. Also, transgenic mice, or other organisms or animals, such as other mammals, may be used to express humanized antibodies immunospecific to the polypeptides or polynucleotides of the invention.

Alternatively, phage display technology may be utilized to select antibody genes with  
15 binding activities towards a polypeptide of the invention either from repertoires of PCR amplified v-genes of lymphocytes from humans screened for possessing anti- BASB047, BASB054, BASB068 or BASB069 or from naive libraries (McCafferty, *et al.*, (1990), *Nature* 348, 552-554; Marks, *et al.*, (1992) *Biotechnology* 10, 779-783). The affinity of these antibodies can also be improved by, for example, chain shuffling (Clackson *et al.*,  
20 (1991) *Nature* 352: 628).

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptides or polynucleotides of the invention to purify the polypeptides or polynucleotides by, for example, affinity chromatography.  
25

Thus, among others, antibodies against BASB047, BASB054, BASB068 or BASB069 - polypeptide or BASB047, BASB054, BASB068 or BASB069 -polynucleotide may be employed to treat infections, particularly bacterial infections.

Polypeptide variants include antigenically, epitopically or immunologically equivalent variants form a particular aspect of this invention.

Preferably, the antibody or variant thereof is modified to make it less immunogenic in the individual. For example, if the individual is human the antibody may most preferably be "humanized," where the complementarity determining region or regions of the hybridoma-derived antibody has been transplanted into a human monoclonal antibody, for example as described in Jones *et al.* (1986), *Nature* 321, 522-525 or Tempest *et al.*, (1991) *Biotechnology* 9, 266-273.

#### Antagonists and Agonists - Assays and Molecules

Polypeptides and polynucleotides of the invention may also be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See, *e.g.*, Coligan *et al.*, *Current Protocols in Immunology* 1(2): Chapter 5 (1991).

The screening methods may simply measure the binding of a candidate compound to the polypeptide or polynucleotide, or to cells or membranes bearing the polypeptide or polynucleotide, or a fusion protein of the polypeptide by means of a label directly or indirectly associated with the candidate compound. Alternatively, the screening method may involve competition with a labeled competitor. Further, these screening methods may test whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide or polynucleotide, using detection systems appropriate to the cells comprising the polypeptide or polynucleotide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed. Constitutively active polypeptide and/or constitutively expressed polypeptides and polynucleotides may be employed in

screening methods for inverse agonists or inhibitors, in the absence of an agonist or inhibitor, by testing whether the candidate compound results in inhibition of activation of the polypeptide or polynucleotide, as the case may be. Further, the screening methods may simply comprise the steps of mixing a candidate compound with a solution  
5 containing a polypeptide or polynucleotide of the present invention, to form a mixture, measuring BASB047, BASB054, BASB068 or BASB069 polypeptide and/or polynucleotide activity in the mixture, and comparing the BASB047, BASB054, BASB068 or BASB069 polypeptide and/or polynucleotide activity of the mixture to a standard. Fusion proteins, such as those made from Fc portion and BASB047, BASB054,  
10 BASB068 or BASB069 polypeptide, as hereinbefore described, can also be used for high-throughput screening assays to identify antagonists of the polypeptide of the present invention, as well as of phylogenetically and and/or functionally related polypeptides (see D. Bennett *et al.*, J Mol Recognition, 8:52-58 (1995); and K. Johanson *et al.*, J Biol Chem, 270(16):9459-9471 (1995)).

15 The polynucleotides, polypeptides and antibodies that bind to and/or interact with a polypeptide of the present invention may also be used to configure screening methods for detecting the effect of added compounds on the production of mRNA and/or polypeptide in cells. For example, an ELISA assay may be constructed for measuring secreted or cell  
20 associated levels of polypeptide using monoclonal and polyclonal antibodies by standard methods known in the art. This can be used to discover agents which may inhibit or enhance the production of polypeptide (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

25 The invention also provides a method of screening compounds to identify those which enhance (agonist) or block (antagonist) the action of BASB047, BASB054, BASB068 or BASB069 polypeptides or polynucleotides, particularly those compounds that are bacteristatic and/or bactericidal. The method of screening may involve high-throughput techniques. For example, to screen for agonists or antagonists, a synthetic reaction mix, a

cellular compartment, such as a membrane, cell envelope or cell wall, or a preparation of any thereof, comprising BASB047, BASB054, BASB068 or BASB069 polypeptide and a labeled substrate or ligand of such polypeptide is incubated in the absence or the presence of a candidate molecule that may be a BASB047, BASB054, BASB068 or BASB069 agonist or antagonist. The ability of the candidate molecule to agonize or antagonize the BASB047, BASB054, BASB068 or BASB069 polypeptide is reflected in decreased binding of the labeled ligand or decreased production of product from such substrate. Molecules that bind gratuitously, *i.e.*, without inducing the effects of BASB047, BASB054, BASB068 or BASB069 polypeptide are most likely to be good antagonists. Molecules that bind well and, as the case may be, increase the rate of product production from substrate, increase signal transduction, or increase chemical channel activity are agonists. Detection of the rate or level of, as the case may be, production of product from substrate, signal transduction, or chemical channel activity may be enhanced by using a reporter system. Reporter systems that may be useful in this regard include but are not limited to colorimetric, labeled substrate converted into product, a reporter gene that is responsive to changes in BASB047, BASB054, BASB068 or BASB069 polynucleotide or polypeptide activity, and binding assays known in the art.

Another example of an assay for BASB047, BASB054, BASB068 or BASB069 agonists is a competitive assay that combines BASB047, BASB054, BASB068 or BASB069 and a potential agonist with BASB047, BASB054, BASB068 or BASB069 -binding molecules, recombinant BASB047, BASB054, BASB068 or BASB069 binding molecules, natural substrates or ligands, or substrate or ligand mimetics, under appropriate conditions for a competitive inhibition assay. BASB047, BASB054, BASB068 or BASB069 can be labeled, such as by radioactivity or a colorimetric compound, such that the number of BASB047, BASB054, BASB068 or BASB069 molecules bound to a binding molecule or converted to product can be determined accurately to assess the effectiveness of the potential antagonist.

Potential antagonists include, among others, small organic molecules, peptides, polypeptides and antibodies that bind to a polynucleotide and/or polypeptide of the invention and thereby inhibit or extinguish its activity or expression. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related  
5 protein or antibody that binds the same sites on a binding molecule, such as a binding molecule, without inducing BASB047, BASB054, BASB068 or BASB069 -induced activities, thereby preventing the action or expression of BASB047, BASB054, BASB068 or BASB069 polypeptides and/or polynucleotides by excluding BASB047, BASB054, BASB068 or BASB069 polypeptides and/or polynucleotides from binding.

10

Potential antagonists include a small molecule that binds to and occupies the binding site of the polypeptide thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented. Examples of small molecules include but are not limited to small organic molecules, peptides or peptide-like molecules. Other potential antagonists  
15 include antisense molecules (see Okano, *J. Neurochem.* 56: 560 (1991); *OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION*, CRC Press, Boca Raton, FL (1988), for a description of these molecules). Preferred potential antagonists include compounds related to and variants of BASB047, BASB054, BASB068 or BASB069.

20

In a further aspect, the present invention relates to genetically engineered soluble fusion proteins comprising a polypeptide of the present invention, or a fragment thereof, and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant  
25 part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa. Furthermore, this invention relates to processes for the preparation of these fusion proteins by genetic engineering, and to the use thereof for drug screening, diagnosis and

therapy. A further aspect of the invention also relates to polynucleotides encoding such fusion proteins. Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

- 5 Each of the polynucleotide sequences provided herein may be used in the discovery and development of antibacterial compounds. The encoded protein, upon expression, can be used as a target for the screening of antibacterial drugs. Additionally, the polynucleotide sequences encoding the amino terminal regions of the encoded protein or Shine-Delgarno or other translation facilitating sequences of the respective mRNA can be used to  
10 construct antisense sequences to control the expression of the coding sequence of interest.

- The invention also provides the use of the polypeptide, polynucleotide, agonist or antagonist of the invention to interfere with the initial physical interaction between a pathogen or pathogens and a eukaryotic, preferably mammalian, host responsible for  
15 sequelae of infection. In particular, the molecules of the invention may be used: in the prevention of adhesion of bacteria, in particular gram positive and/or gram negative bacteria, to eukaryotic, preferably mammalian, extracellular matrix proteins on in-dwelling devices or to extracellular matrix proteins in wounds; to block bacterial adhesion between eukaryotic, preferably mammalian, extracellular matrix proteins and bacterial  
20 BASB047, BASB054, BASB068 or BASB069 proteins that mediate tissue damage and/or; to block the normal progression of pathogenesis in infections initiated other than by the implantation of in-dwelling devices or by other surgical techniques.

- In accordance with yet another aspect of the invention, there are provided BASB047,  
25 BASB054, BASB068 or BASB069 agonists and antagonists, preferably bacteristatic or bactericidal agonists and antagonists.

The antagonists and agonists of the invention may be employed, for instance, to prevent, inhibit and/or treat diseases.



In a further aspect, the present invention relates to mimotopes of the polypeptide of the invention. A mimotope is a peptide sequence, sufficiently similar to the native peptide (sequentially or structurally), which is capable of being recognised by antibodies which  
5 recognise the native peptide; or is capable of raising antibodies which recognise the native peptide when coupled to a suitable carrier.

Peptide mimotopes may be designed for a particular purpose by addition, deletion or substitution of elected amino acids. Thus, the peptides may be modified for the purposes  
10 of ease of conjugation to a protein carrier. For example, it may be desirable for some chemical conjugation methods to include a terminal cysteine. In addition it may be desirable for peptides conjugated to a protein carrier to include a hydrophobic terminus distal from the conjugated terminus of the peptide, such that the free unconjugated end of the peptide remains associated with the surface of the carrier protein. Thereby  
15 presenting the peptide in a conformation which most closely resembles that of the peptide as found in the context of the whole native molecule. For example, the peptides may be altered to have an N-terminal cysteine and a C-terminal hydrophobic amidated tail. Alternatively, the addition or substitution of a D-stereoisomer form of one or more of the amino acids may be performed to create a beneficial derivative, for example to  
20 enhance stability of the peptide.

Alternatively, peptide mimotopes may be identified using antibodies which are capable themselves of binding to the polypeptides of the present invention using techniques such as phage display technology (EP 0 552 267 B1). This technique, generates a large number  
25 of peptide sequences which mimic the structure of the native peptides and are, therefore, capable of binding to anti-native peptide antibodies, but may not necessarily themselves share significant sequence homology to the native polypeptide.

### Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in an individual, particularly a mammal, preferably humans, which comprises inoculating the individual with BASB047, BASB054, BASB068 or BASB069

5 polynucleotide and/or polypeptide, or a fragment or variant thereof, adequate to produce antibody and/ or T cell immune response to protect said individual from infection, particularly bacterial infection and most particularly *Neisseria meningitidis* infection.

Also provided are methods whereby such immunological response slows bacterial replication. Yet another aspect of the invention relates to a method of inducing

10 immunological response in an individual which comprises delivering to such individual a nucleic acid vector, sequence or ribozyme to direct expression of BASB047, BASB054, BASB068 or BASB069 polynucleotide and/or polypeptide, or a fragment or a variant thereof, for expressing BASB047, BASB054, BASB068 or BASB069 polynucleotide and/or polypeptide, or a fragment or a variant thereof *in vivo* in order to induce an

15 immunological response, such as, to produce antibody and/ or T cell immune response, including, for example, cytokine-producing T cells or cytotoxic T cells, to protect said individual, preferably a human, from disease, whether that disease is already established within the individual or not. One example of administering the gene is by accelerating it into the desired cells as a coating on particles or otherwise. Such nucleic acid vector may

20 comprise DNA, RNA, a ribozyme, a modified nucleic acid, a DNA/RNA hybrid, a DNA-protein complex or an RNA-protein complex.

A further aspect of the invention relates to an immunological composition that when introduced into an individual, preferably a human, capable of having induced within it an

25 immunological response, induces an immunological response in such individual to a BASB047, BASB054, BASB068 or BASB069 polynucleotide and/or polypeptide encoded therefrom, wherein the composition comprises a recombinant BASB047, BASB054, BASB068 or BASB069 polynucleotide and/or polypeptide encoded therefrom and/or comprises DNA and/or RNA which encodes and expresses an antigen of said BASB047.

BASB054, BASB068 or BASB069 polynucleotide, polypeptide encoded therefrom, or other polypeptide of the invention. The immunological response may be used therapeutically or prophylactically and may take the form of antibody immunity and/or cellular immunity, such as cellular immunity arising from CTL or CD4+ T cells.

5

A BASB047, BASB054, BASB068 or BASB069 polypeptide or a fragment thereof may be fused with co-protein or chemical moiety which may or may not by itself produce antibodies, but which is capable of stabilizing the first protein and producing a fused or modified protein which will have antigenic and/or immunogenic properties, and preferably protective properties. Thus fused recombinant protein, preferably further comprises an antigenic co-protein, such as lipoprotein D from *Haemophilus influenzae*, Glutathione-S-transferase (GST) or beta-galactosidase, or any other relatively large co-protein which solubilizes the protein and facilitates production and purification thereof. Moreover, the co-protein may act as an adjuvant in the sense of providing a generalized stimulation of the immune system of the organism receiving the protein. The co-protein may be attached to either the amino- or carboxy-terminus of the first protein.

10  
15

In a vaccine composition according to the invention, a BASB047, BASB054, BASB068 or BASB069 polypeptide and/or polynucleotide, or a fragment, or a mimotope, or a variant thereof may be present in a vector, such as the live recombinant vectors described above for example live bacterial vectors.

20

Also suitable are non-live vectors for the BASB047, BASB054, BASB068 or BASB069 polypeptide, for example bacterial outer-membrane vesicles or "blebs". OM blebs are derived from the outer membrane of the two-layer membrane of Gram-negative bacteria and have been documented in many Gram-negative bacteria (Zhou, L *et al.* 1998. *FEMS Microbiol. Lett.* 163:223-228) including *C. trachomatis* and *C. psittaci*. A non-exhaustive list of bacterial pathogens reported to produce blebs also includes: *Bordetella pertussis*, *Borrelia burgdorferi*, *Brucella melitensis*, *Brucella ovis*, *Escherichia coli*, *Haemophilus*

25

*influenza, Legionella pneumophila, Neisseria gonorrhoeae, Neisseria meningitidis, Pseudomonas aeruginosa and Yersinia enterocolitica.*

- Blebs have the advantage of providing outer-membrane proteins in their native
- 5 conformation and are thus particularly useful for vaccines. Blebs can also be improved for vaccine use by engineering the bacterium so as to modify the expression of one or more molecules at the outer membrane. Thus for example the expression of a desired immunogenic protein at the outer membrane, such as the BASB047, BASB054, BASB068 or BASB069 polypeptide, can be introduced or upregulated (e.g. by altering the
- 10 promoter). Instead or in addition, the expression of outer-membrane molecules which are either not relevant (e.g. unprotective antigens or immunodominant but variable proteins) or detrimental (e.g. toxic molecules such as LPS, or potential inducers of an autoimmune response) can be downregulated. These approaches are discussed in more detail below.
- 15 The non-coding flanking regions of the BASB047, BASB054, BASB068 or BASB069 gene contain regulatory elements important in the expression of the gene. This regulation takes place both at the transcriptional and translational level. The sequence of these regions, either upstream or downstream of the open reading frame of the gene, can be obtained by DNA sequencing. This sequence information allows the determination of
- 20 potential regulatory motifs such as the different promoter elements, terminator sequences, inducible sequence elements, repressors, elements responsible for phase variation, the shine-dalgarno sequence, regions with potential secondary structure involved in regulation, as well as other types of regulatory motifs or sequences.
- 25 This sequence information allows the modulation of the natural expression of the BASB047, BASB054, BASB068 or BASB069 gene. The upregulation of the gene expression may be accomplished by altering the promoter, the shine-dalgarno sequence, potential repressor or operator elements, or any other elements involved. Likewise, downregulation of expression can be achieved by similar types of modification.

Alternatively, by changing phase variation sequences, the expression of the gene can be put under phase variation control, or it may be uncoupled from this regulation. In another approach, the expression of the gene can be put under the control of one or more inducible elements allowing regulated expression. Examples of such regulation include, but are not  
5 limited to, induction by temperature shift, addition of inductor substrates like selected carbohydrates or their derivatives, trace elements, vitamins, co-factors, metal ions, etc.

Such modifications as described above can be introduced by several different means. The modification of sequences involved in gene expression can be carried out *in vivo* by  
10 random mutagenesis followed by selection for the desired phenotype. Another approach consists in isolating the region of interest and modifying it by random mutagenesis, or site-directed replacement, insertion or deletion mutagenesis. The modified region can then be reintroduced into the bacterial genome by homologous recombination, and the effect on gene expression can be assessed. In another approach, the sequence knowledge of the  
15 region of interest can be used to replace or delete all or part of the natural regulatory sequences. In this case, the regulatory region targeted is isolated and modified so as to contain the regulatory elements from another gene, a combination of regulatory elements from different genes, a synthetic regulatory region, or any other regulatory region, or to delete selected parts of the wild-type regulatory sequences. These modified sequences can  
20 then be reintroduced into the bacterium via homologous recombination into the genome. A non-exhaustive list of preferred promoters that could be used for up-regulation of gene expression includes the promoters *porA*, *porB*, *lbpB*, *tbpB*, *p110*, *lst*, *hpuAB* from *N. meningitidis* or *N. gonorrhoeae*; *ompCD*, *copB*, *lbpB*, *ompE*, *UspA1*; *UspA2*; *TbpB* from *M. Catarrhalis*; *p1*, *p2*, *p4*, *p5*, *p6*, *lpD*, *tbpB*, *D15*, *Hia*, *Hmw1*, *Hmw2* from *H.*  
25 *influenzae*.

In one example, the expression of the gene can be modulated by exchanging its promoter with a stronger promoter (through isolating the upstream sequence of the gene, *in vitro* modification of this sequence, and reintroduction into the genome by homologous

recombination). Upregulated expression can be obtained in both the bacterium as well as in the outer membrane vesicles shed (or made) from the bacterium.

5 In other examples, the described approaches can be used to generate recombinant bacterial strains with improved characteristics for vaccine applications. These can be, but are not limited to, attenuated strains, strains with increased expression of selected antigens, strains with knock-outs (or decreased expression) of genes interfering with the immune response, strains with modulated expression of immunodominant proteins, strains with modulated shedding of outer-membrane vesicles.

10 Thus, also provided by the invention is a modified upstream region of the BASB047, BASB054, BASB068 or BASB069 gene, which modified upstream region contains a heterologous regulatory element which alters the expression level of the BASB047, BASB054, BASB068 or BASB069 protein located at the outer membrane. The upstream  
15 region according to this aspect of the invention includes the sequence upstream of the BASB047, BASB054, BASB068 or BASB069 gene. The upstream region starts immediately upstream of the BASB047, BASB054, BASB068 or BASB069 gene and continues usually to a position no more than about 1000 bp upstream of the gene from the ATG start codon. In the case of a gene located in a polycistronic sequence (operon) the  
20 upstream region can start immediately preceding the gene of interest, or preceding the first gene in the operon. Preferably, a modified upstream region according to this aspect of the invention contains a heterologous promotor at a position between 500 and 700 bp upstream of the ATG.

25 Thus, the invention provides a BASB047, BASB054, BASB068 and BASB069 polypeptide, in a modified bacterial bleb. The invention further provides modified host cells capable of producing the non-live membrane-based bleb vectors. The invention further provides nucleic acid vectors comprising the BASB047, BASB054, BASB068 and

BASB069 gene having a modified upstream region containing a heterologous regulatory element.

5 Further provided by the invention are processes to prepare the host cells and bacterial blebs according to the invention.

Also provided by this invention are compositions, particularly vaccine compositions, and methods comprising the polypeptides and/or polynucleotides of the invention and immunostimulatory DNA sequences, such as those described in Sato, Y. *et al.* Science  
10 273: 352 (1996).

Also, provided by this invention are methods using the described polynucleotide or particular fragments thereof, which have been shown to encode non-variable regions of bacterial cell surface proteins, in polynucleotide constructs used in such genetic  
15 immunization experiments in animal models of infection with *Neisseria meningitidis*. Such experiments will be particularly useful for identifying protein epitopes able to provoke a prophylactic or therapeutic immune response. It is believed that this approach will allow for the subsequent preparation of monoclonal antibodies of particular value, derived from the requisite organ of the animal successfully resisting or clearing infection,  
20 for the development of prophylactic agents or therapeutic treatments of bacterial infection, particularly *Neisseria meningitidis* infection, in mammals, particularly humans.

The invention also includes a vaccine formulation which comprises an immunogenic recombinant polypeptide and/or polynucleotide of the invention together with a suitable  
25 carrier, such as a pharmaceutically acceptable carrier. Since the polypeptides and polynucleotides may be broken down in the stomach, each is preferably administered parenterally, including, for example, administration that is subcutaneous, intramuscular, intravenous, or intradermal. Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants.

5 buffers, bacteristatic compounds and solutes which render the formulation isotonic with the bodily fluid, preferably the blood, of the individual; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use.

10 The vaccine formulation of the invention may also include adjuvant systems for enhancing the immunogenicity of the formulation. Preferably the adjuvant system raises preferentially a TH1 type of response.

15 An immune response may be broadly distinguished into two extreme categories, being a humoral or cell mediated immune responses (traditionally characterised by antibody and cellular effector mechanisms of protection respectively). These categories of response have been termed TH1-type responses (cell-mediated response), and TH2-type immune responses (humoral response).

20 Extreme TH1-type immune responses may be characterised by the generation of antigen specific, haplotype restricted cytotoxic T lymphocytes, and natural killer cell responses. In mice TH1-type responses are often characterised by the generation of antibodies of the IgG2a subtype, whilst in the human these correspond to IgG1 type antibodies. TH2-type immune responses are characterised by the generation of a broad range of immunoglobulin isotypes including in mice IgG1, IgA, and IgM.

25 It can be considered that the driving force behind the development of these two types of immune responses are cytokines. High levels of TH1-type cytokines tend to favour the induction of cell mediated immune responses to the given antigen, whilst high levels of TH2-type cytokines tend to favour the induction of humoral immune responses to the antigen.



The distinction of TH1 and TH2-type immune responses is not absolute. In reality an individual will support an immune response which is described as being predominantly TH1 or predominantly TH2. However, it is often convenient to consider the families of cytokines in terms of that described in murine CD4 +ve T cell clones by Mosmann and Coffman (*Mosmann, T.R. and Coffman, R.L. (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annual Review of Immunology, 7, p145-173*). Traditionally, TH1-type responses are associated with the production of the INF- $\gamma$  and IL-2 cytokines by T-lymphocytes. Other cytokines often directly associated with the induction of TH1-type immune responses are not produced by T-cells, such as IL-12. In contrast, TH2-type responses are associated with the secretion of IL-4, IL-5, IL-6 and IL-13.

It is known that certain vaccine adjuvants are particularly suited to the stimulation of either TH1 or TH2 - type cytokine responses. Traditionally the best indicators of the TH1:TH2 balance of the immune response after a vaccination or infection includes direct measurement of the production of TH1 or TH2 cytokines by T lymphocytes *in vitro* after restimulation with antigen, and/or the measurement of the IgG1:IgG2a ratio of antigen specific antibody responses.

Thus, a TH1-type adjuvant is one which preferentially stimulates isolated T-cell populations to produce high levels of TH1-type cytokines when re-stimulated with antigen *in vitro*, and promotes development of both CD8+ cytotoxic T lymphocytes and antigen specific immunoglobulin responses associated with TH1-type isotype.

Adjuvants which are capable of preferential stimulation of the TH1 cell response are described in International Patent Application No. WO 94/00153 and WO 95/17209.

3 De-O-acylated monophosphoryl lipid A (3D-MPL) is one such adjuvant. This is known from GB 2220211 (Ribi). Chemically it is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi Immunochem, Montana. A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in European Patent 0 689 454 B1 (SmithKline Beecham Biologicals SA).

Preferably, the particles of 3D-MPL are small enough to be sterile filtered through a 0.22micron membrane (European Patent number 0 689 454).

3D-MPL will be present in the range of 10 $\mu$ g - 100 $\mu$ g preferably 25-50 $\mu$ g per dose wherein the antigen will typically be present in a range 2-50 $\mu$ g per dose.

Another preferred adjuvant comprises QS21, an Hplc purified non-toxic fraction derived from the bark of Quillaja Saponaria Molina. Optionally this may be admixed with 3 De-O-acylated monophosphoryl lipid A (3D-MPL), optionally together with a carrier.

The method of production of QS21 is disclosed in US patent No. 5,057,540.

Non-reactogenic adjuvant formulations containing QS21 have been described previously (WO 96/33739). Such formulations comprising QS21 and cholesterol have been shown to be successful TH1 stimulating adjuvants when formulated together with an antigen.

Further adjuvants which are preferential stimulators of TH1 cell response include immunomodulatory oligonucleotides, for example unmethylated CpG sequences as disclosed in WO 96/02555.

Combinations of different TH1 stimulating adjuvants, such as those mentioned hereinabove, are also contemplated as providing an adjuvant which is a preferential

stimulator of TH1 cell response. For example, QS21 can be formulated together with 3D-MPL. The ratio of QS21 : 3D-MPL will typically be in the order of 1 : 10 to 10 : 1; preferably 1:5 to 5 : 1 and often substantially 1 : 1. The preferred range for optimal synergy is 2.5 : 1 to 1 : 1 3D-MPL: QS21.

5

Preferably a carrier is also present in the vaccine composition according to the invention. The carrier may be an oil in water emulsion, or an aluminium salt, such as aluminium phosphate or aluminium hydroxide.

- 10 A preferred oil-in-water emulsion comprises a metabolisable oil, such as squalene, alpha tocopherol and Tween 80. In a particularly preferred aspect the antigens in the vaccine composition according to the invention are combined with QS21 and 3D-MPL in such an emulsion. Additionally the oil in water emulsion may contain span 85 and/or lecithin and/or tricaprylin.

15

Typically for human administration QS21 and 3D-MPL will be present in a vaccine in the range of 1µg - 200µg, such as 10-100µg, preferably 10µg - 50µg per dose.

- Typically the oil in water will comprise from 2 to 10% squalene, from 2 to 10% alpha tocopherol and from 0.3 to 3% tween 80. Preferably the ratio of squalene: alpha  
20 tocopherol is equal to or less than 1 as this provides a more stable emulsion. Span 85 may also be present at a level of 1%. In some cases it may be advantageous that the vaccines of the present invention will further contain a stabiliser.

- 25 Non-toxic oil in water emulsions preferably contain a non-toxic oil, e.g. squalane or squalene, an emulsifier, e.g. Tween 80, in an aqueous carrier. The aqueous carrier may be, for example, phosphate buffered saline.

A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil in water emulsion is described in WO 95/17210.

The present invention also provides a polyvalent vaccine composition comprising a vaccine formulation of the invention in combination with other antigens, in particular antigens useful for treating cancers, autoimmune diseases and related conditions. Such a  
5 polyvalent vaccine composition may include a TH-1 inducing adjuvant as hereinbefore described.

While the invention has been described with reference to certain BASB047, BASB054, BASB068 and BASB069 polypeptides and polynucleotides, it is to be understood that this  
10 covers fragments of the naturally occurring polypeptides and polynucleotides, and similar polypeptides and polynucleotides with additions, deletions or substitutions which do not substantially affect the immunogenic properties of the recombinant polypeptides or polynucleotides.

15 The antigen can also be delivered in the form of whole bacteria (dead or alive) or as subcellular fractions, these possibilities do include *N.meningitidis* itself.

#### Compositions, kits and administration

20 In a further aspect of the invention there are provided compositions comprising a BASB047, BASB054, BASB068 or BASB069 polynucleotide and/or a BASB047, BASB054, BASB068 or BASB069 polypeptide for administration to a cell or to a multicellular organism.

25 The invention also relates to compositions comprising a polynucleotide and/or a polypeptide discussed herein or their agonists or antagonists. The polypeptides and polynucleotides of the invention may be employed in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to an individual. Such compositions comprise, for instance, a media additive

or a therapeutically effective amount of a polypeptide and/or polynucleotide of the invention and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode of administration. The invention further  
5 relates to diagnostic and pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides, polynucleotides and other compounds of the invention may be employed  
10 alone or in conjunction with other compounds, such as therapeutic compounds.

The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.  
15

In therapy or as a prophylactic, the active agent may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic.

20 In a further aspect, the present invention provides for pharmaceutical compositions comprising a therapeutically effective amount of a polypeptide and/or polynucleotide, such as the soluble form of a polypeptide and/or polynucleotide of the present invention, agonist or antagonist peptide or small molecule compound, in combination with a pharmaceutically acceptable carrier or excipient. Such carriers include, but are not limited to, saline, buffered  
25 saline, dextrose, water, glycerol, ethanol, and combinations thereof. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides, polynucleotides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

The composition will be adapted to the route of administration, for instance by a systemic or an oral route. Preferred forms of systemic administration include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if a polypeptide or other compounds of the present invention can be formulated in an enteric or an encapsulated formulation, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels, solutions, powders and the like.

For administration to mammals, and particularly humans, it is expected that the daily dosage level of the active agent will be from 0.01 mg/kg to 10 mg/kg, typically around 1 mg/kg. The physician in any event will determine the actual dosage which will be most suitable for an individual and will vary with the age, weight and response of the particular individual. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100  $\mu$ g/kg of subject.

A vaccine composition is conveniently in injectable form. Conventional adjuvants may be employed to enhance the immune response. A suitable unit dose for vaccination is 0.5-5 microgram/kg of antigen, and such dose is preferably administered 1-3 times and with an interval of 1-3 weeks. With the indicated dose range, no adverse toxicological effects will

be observed with the compounds of the invention which would preclude their administration to suitable individuals.

5 Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

10 **Sequence Databases, Sequences in a Tangible Medium, and Algorithms**

Polynucleotide and polypeptide sequences form a valuable information resource with which to determine their 2- and 3-dimensional structures as well as to identify further sequences of similar homology. These approaches are most easily facilitated by storing the sequence in a  
15 computer readable medium and then using the stored data in a known macromolecular structure program or to search a sequence database using well known searching tools, such as the GCG program package.

Also provided by the invention are methods for the analysis of character sequences or  
20 strings, particularly genetic sequences or encoded protein sequences. Preferred methods of sequence analysis include, for example, methods of sequence homology analysis, such as identity and similarity analysis, DNA, RNA and protein structure analysis, sequence assembly, cladistic analysis, sequence motif analysis, open reading frame determination, nucleic acid base calling, codon usage analysis, nucleic acid base trimming, and  
25 sequencing chromatogram peak analysis.

A computer based method is provided for performing homology identification. This method comprises the steps of: providing a first polynucleotide sequence comprising the sequence of a polynucleotide of the invention in a computer readable medium; and

comparing said first polynucleotide sequence to at least one second polynucleotide or polypeptide sequence to identify homology.

5 A computer based method is also provided for performing homology identification, said method comprising the steps of: providing a first polypeptide sequence comprising the sequence of a polypeptide of the invention in a computer readable medium; and comparing said first polypeptide sequence to at least one second polynucleotide or polypeptide sequence to identify homology.

10 All publications and references, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference in their entirety as if each individual publication or reference were specifically and individually indicated to be incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference  
15 herein in its entirety in the manner described above for publications and references.

## DEFINITIONS

"Identity," as known in the art, is a relationship between two or more polypeptide sequences  
20 or two or more polynucleotide sequences, as the case may be, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" can be readily calculated by known methods, including but not limited to those described in (*Computational Molecular*  
25 *Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heine, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J.,



- eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., *SIAM J. Applied Math.*, 48: 1073 (1988). Methods to determine identity are designed to give the largest match between the sequences tested. Moreover, methods to determine identity are codified in publicly available computer programs. Computer program methods to
- 5 determine identity between two sequences include, but are not limited to, the GAP program in the GCG program package (Devereux, J., et al., *Nucleic Acids Research* 12(1): 387 (1984)), BLASTP, BLASTN (Altschul, S.F. et al., *J. Mol. Biol.* 215: 403-410 (1990), and FASTA( Pearson and Lipman Proc. Natl. Acad. Sci. USA 85: 2444-2448 (1988). The BLAST family of programs is publicly available from NCBI and other sources
- 10 (*BLAST Manual*, Altschul, S., et al., NCBI NLM NIH Bethesda, MD 20894; Altschul, S., et al., *J. Mol. Biol.* 215: 403-410 (1990). The well known Smith Waterman algorithm may also be used to determine identity.

Parameters for polypeptide sequence comparison include the following:

- 15 Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)  
Comparison matrix: BLOSSUM62 from Henikoff and Henikoff,  
*Proc. Natl. Acad. Sci. USA.* 89:10915-10919 (1992)  
Gap Penalty: 8  
Gap Length Penalty: 2
- 20 A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for peptide comparisons (along with no penalty for end gaps).

Parameters for polynucleotide comparison include the following:

- 25 Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)  
Comparison matrix: matches = +10, mismatch = 0  
Gap Penalty: 50  
Gap Length Penalty: 3

Available as: The "gap" program from Genetics Computer Group, Madison WI. These are the default parameters for nucleic acid comparisons.

5 A preferred meaning for "identity" for polynucleotides and polypeptides, as the case may be, are provided in (1) and (2) below.

(1) Polynucleotide embodiments further include an isolated polynucleotide comprising a polynucleotide sequence having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100% identity to the reference sequence of SEQ ID NO:1, wherein said polynucleotide sequence may be identical to the reference sequence of SEQ ID NO:1 or may include up to a certain integer number of nucleotide alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleotides in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \cdot y),$$

25 wherein  $n_n$  is the number of nucleotide alterations,  $x_n$  is the total number of nucleotides in SEQ ID NO:1,  $y$  is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and  $\cdot$  is the symbol for the multiplication operator, and wherein any non-integer product of  $x_n$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_n$ . Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or

frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

By way of example, a polynucleotide sequence of the present invention may be identical  
5 to the reference sequence of SEQ ID NO:1, that is it may be 100% identical, or it may include up to a certain integer number of nucleic acid alterations as compared to the reference sequence such that the percent identity is less than 100% identity. Such alterations are selected from the group consisting of at least one nucleic acid deletion, substitution, including transition and transversion, or insertion, and wherein said  
10 alterations may occur at the 5' or 3' terminal positions of the reference polynucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleic acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleic acid alterations for a given percent identity is determined by multiplying the total number of nucleic acids in SEQ ID NO:1  
15 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleic acids in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \cdot y),$$

20 wherein  $n_n$  is the number of nucleic acid alterations,  $x_n$  is the total number of nucleic acids in SEQ ID NO:1,  $y$  is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc.,  $\cdot$  is the symbol for the multiplication operator, and wherein any non-integer product of  $x_n$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_n$ .

25 (2) Polypeptide embodiments further include an isolated polypeptide comprising a polypeptide having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100% identity to a polypeptide reference sequence of SEQ ID NO:2, wherein said polypeptide sequence may be identical to the reference sequence of SEQ ID NO:2 or may include up to a certain integer number of amino acid alterations as compared to the reference sequence, wherein

said alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions,

5 interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of amino acid alterations is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

10

$$n_a \leq x_a - (x_a \bullet y),$$

wherein  $n_a$  is the number of amino acid alterations,  $x_a$  is the total number of amino acids in SEQ ID NO:2,  $y$  is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 15 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and  $\bullet$  is the symbol for the multiplication operator, and wherein any non-integer product of  $x_a$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_a$ .

By way of example, a polypeptide sequence of the present invention may be identical to 20 the reference sequence of SEQ ID NO:2, that is it may be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the percent identity is less than 100% identity. Such alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and 25 wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined by multiplying the total number of amino

acids in SEQ ID NO:2 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \bullet y),$$

5

wherein  $n_a$  is the number of amino acid alterations,  $x_a$  is the total number of amino acids in SEQ ID NO:2,  $y$  is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., and  $\bullet$  is the symbol for the multiplication operator, and wherein any non-integer product of  $x_a$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_a$ .

10

"Individual(s)," when used herein with reference to an organism, means a multicellular eukaryote, including, but not limited to a metazoan, a mammal, an ovid, a bovid, a simian, a primate, and a human.

15 "Isolated" means altered "by the hand of man" from its natural state, *i.e.*, if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. Moreover, a polynucleotide or  
20 polypeptide that is introduced into an organism by transformation, genetic manipulation or by any other recombinant method is "isolated" even if it is still present in said organism, which organism may be living or non-living.

25 "Polynucleotide(s)" generally refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA including single and double-stranded regions.

"Variant" refers to a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide, but retains essential properties. A typical variant of a

polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Disease(s)" means any disease caused by or related to infection by a bacteria, including , for example, upper respiratory tract infection, invasive bacterial diseases, such as bacteremia and meningitis.

## EXAMPLES

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples  
5 are illustrative, but do not limit the invention.

### Example 1

#### The BASB047 gene in *N.meningitidis* strain ATCC 13090.

10

The BASB047 gene of *N. meningitidis* strain ATCC 13090 is shown in SEQ ID NO:1. The translation of the BASB047 polynucleotide sequence, shown in SEQ ID NO:2, shows significant similarity to *E. coli* ferric aerobactin receptor precursor (cloacin receptor). The BASB047 polypeptide contains a sequence which has the  
15 characteristics of a leader signal sequence. The signal sequence would be cleaved after residue 25 of the sequence shown in SEQ ID NO:2. BASB047 has the characteristics of an outer membrane protein involved iron uptake.

### Example 2

20

#### The BASB054 gene in *N.meningitidis* strain ATCC 13090.

The BASB054 gene of *N. meningitidis* strain ATCC 13090 is shown in SEQ ID NO:3. The translation of the BASB054 polynucleotide sequence, shown in SEQ ID  
25 NO:4, shows significant similarity to a *E. coli* organic solvent tolerance protein. The BASB054 polypeptide contains a sequence which has the characteristics of a leader signal sequence. This signal sequence would be cleaved after residue 22 of the SEQ ID NO:4 polypeptide.

**Example 3****The BASB068 gene in *N.meningitidis* strain ATCC 13090.**

- 5 The BASB068 gene of *N. meningitidis* strain ATCC 13090 is shown in SEQ ID NO:5. The translation of the BASB068 polynucleotide sequence, shown in SEQ ID NO:6, shows significant similarity to *E. coli* AIDA-I protein. The BASB068 polypeptide contains a sequence which has the characteristics of a leader signal sequence. This signal sequence would be cleaved after residue 34 of the polypeptide  
10 in SEQ ID NO:6. BASB068 has the characteristics of an outer membrane protein.

**Example 4****The BASB069 gene in *N.meningitidis* strain ATCC 13090.**

- 15 The BASB069 gene of *N. meningitidis* strain ATCC 13090 is shown in SEQ ID NO:7. The translation of the BASB069 polynucleotide sequence, shown in SEQ ID NO:8, shows significant similarity to *E coli* AIDA-I protein. The BASB069 polypeptide contains a sequence which has the characteristics of a leader signal  
20 sequence. This signal sequence would be cleaved after residue 31 of the sequence of SEQ ID NO:8. BASB069 has the characteristics of an outer membrane protein.



## Polynucleotide and Polypeptide Sequences

### SEQ ID NO:1

*Neisseria meningitidis* BASB047 polynucleotide sequence from strain ATCC 13090

```

5  ATGCGTCATTCCCACTATTTTCAATGGTTATCTTTGCCTTTACTAAGTGTGGCAGTAACT
   CAGCAGTTGTACGCTCAACCCAATGAGTCATTACCAACGGTTGAATTAGAGCCTGTGGTT
   ATTACCATTGATAAGAGCGGTATGGCACTTGCCAATCGTATCACGCAAATGCCCCATACC
   ACCAAAGTTATTTATGAAGAGCAAATTCAAGAGCAAGCAACAGGCTCTCGACAGCTTGCC
10  GATGTGATGGCACAGCTCATTCCAAGTTTGGGGTAAGTAGTGGCACTACCAGTAACTTT
   GGGCAAACCATGCACGGTCGTCAAGTGCAATTTTGTAAATGGCGTGCCTTTGACAGGT
   TCGCGAGACATCTCTAGACAGCTTAATAGTATCAATCCCAATCAAGTGGCTAGAATTGAA
   GTTTTATCAGGAGCAACCAGTATTTATGGGTCTGGAGCAACAGGCGGTTTGATTAATATC
   GTTACTAAGTCTGATTTGGAAGAGGAGCAATTTGAAACCCGCATCGGTGTACATGGTAGT
15  AAATTATCCAGTGAAGGTATCGGTTATCAGGTAGGTGAGAGTGTAGCAGGTGTGAGCGAA
   AATGGTAAATGTCTTGCACGACTTGATGTGACTATCGCACCACAGGAGGGGCATTTGAT
   GCTAACGGTAAACGCATCGCTCCTGAGCCTGCCCAAAGTATAAGCAAGACAGCAAAGC
   CTAAGTGTCAATACAAATGTTGATTGGCAACTTGACGACAAGCAAAATATCAATCTGGCA
   TTGACGCATTATAACGACAAACAAGATACCGATTATGCACCTGATTATGGTAATCGCCTT
20  GCGGTGTTGTTTGGAGAAAAGCCTTCATTAAATGCCATCAAAGGCTTATCATTATCAGAA
   CAGCCAAAACCAACCAAAAGCACCTTTAATATCAACTATCATCATGATGATTTGTGGGGT
   AACACCATCAATACCAATGCTTATTATCGCAGAGAGAAAGGCAGATTTTATCCCTTTGTT
   GCCCCGTTTTTCGATCGCCAAAGCCCTGCCTATTTTACAAAGCATGAATTTGCCATCAGCC
25  ACTTTGGATGCTTATACCAAGGCTCCACAAGCTCGCGCCTATGGGGTGTTACAATCCGAA
   TCTAAGGCAGAGGTACTAGGGCGTGTCCTAATTTGAATAAGCCCAAAGAGCCCTATTT
   TAA

```

### SEQ ID NO:2

*Neisseria meningitidis* BASB047 polypeptide sequence deduced from the polynucleotide sequence of SEQ ID NO:1

```

30  MRHSHYFQWLSLPLLSVAVTQQLYAQPNESLPTVELEPVVITIDKSGMALANRITQMPHT
   TKVIYEEQIQEQATGSRQLADVMAQLIPSLGVSSGTTSNFGQTMHGRQVQFLNGVPLTG
   SRDISRQLNSINPNQVARIEVLGATSIIYGSGATGGLINIVTKSDLEEEQFETRIGVHGS
35  KLSSEGIGYQVGQSVAGVSENGNVLARLDVDYRTTGGAFFDANGKRIAPPAQTDKQDSKS
   LSVNTNVDWQLDDKQINLALTHYNDKQDQTDYAPDYGNRLAVLFGEKPSLNAIKGLSLSE
   QPKTTKSTFNINYHDDDLWGNTINTNAYYRREKGRFYFPFVAPFSIAKALPILQSMNLP
   TLDAYTKAPQARAYGVLSSESKEVLGRVPLNPKRALF

```

### SEQ ID NO:3

*Neisseria meningitidis* BASB054 polynucleotide sequence from strain ATCC 13090

```

40  TTGGCTCGTTTATTTTCACTCAAACCACTGGTGCTGGCATTGGGCTTCTGCTTCGGCAGC
   CATTGCGCCGCCGCCGATGCCGTTGCGGCGGAGGAAACGGACAATCCGACCGCCGGAGGA
   AGTGTTCGGAGCGTGTCCGAACCCATGCAGCCTGCCGGCCTGAGCCTCGGTTTCGACCTGC
   CTGTTTTGCAGTAACGAAAGCGGCAAACCCGAAAAAACCGAATCTGCCGTCAAAGGAAGC

```

5 GGCGAAGGGCCTGTGCCCCGAAAACACACGCGAATTGTGCGCCGACAAGGTGGAAGGGCAG  
 TCGCAGGTCAAGGTACGCGCGGAGGGCGGCGTCGTTGTGGAACGCAACCGGACGACCCCTT  
 AATGCCGACTGGGCGGATTACGACCAGTCGGGCGACACCGTTACCGTAGGCGACCGGTTTC  
 10 GCCCTCCAACAGGACGGTACGCTGATTGCGGGCGAAACCCTGACCTACAATCTCGAGCAG  
 CAGACCGGCGAAGCGCACAACTCCGCATGGAAACCGAACAAGGCGGACGGCGGCTGCAA  
 AGCGTCAGCCGACCGCCGAAATGTTGGGCGAAGGGCATTACAACTGACGGAAACCCAA  
 TTCAACACCTGTTCCGCCGCGCATGCCGGCTGGTATGTCAAGGCAGCCTCTGTGGAAGCC  
 GATCGGGAAAAAGGCATAGGCGTTGCCAAACACGCCGCTTTCGTGTTGCGCGGCGTTCTT  
 15 ATTTTCTACACCCCTTGGGCGGACTTCCCGCTTGACGGCAACCGCAAAAGCGGCCTGCTT  
 GTTCCCTCACTGTCCGCCGTTTCGGACGGCGTTTCCCTTTCCGTTCCCTATTATTTCAAC  
 CTTGCCCCCAATCTCGATGCCACGTTTCGCGCCAGCGTGATCGGCGAACGCGGCGCGGTC  
 TTTGACGGGCGAGGTACGCTACCTGCGGCGGATTATGCCGGCCAGTCCGACCTGACCTGG  
 CTGCCGCACGACAAGAAAAGCGGCAGGAATAACCGCTATCAGGCGAAATGGCAGCATCGG  
 CACGACATTTCCGACACGCTTCAGGCGGGTGTGATTTCAACCAAGTCTCCGACAGCGGC  
 20 TACTACCGCGACTTTTACGGCAACAAAGAAATCGCCGGCAACGTCAACCTCAACCGCCGT  
 GTATGGCTGGATTATGGCGGCAGGGCGGGCGGCGAGCCTGAATGCCGGCCTTTTCGGTT  
 CTGAAATACCAGACGCTGGCAAACCAAAGCGGCTACAAAGACAAACCGTATGCCCTGATG  
 CCGCGCCTTTCCGCCGATTGGCGCAAAACACCGCGAGGGCGCAAATCGGCGTGTCCGCA  
 CAATTTACCCGCTTCAGCCACGACAGCCGCCAAGACGGCAGCCGCTCGTCTATATCCC  
 25 GACATCAAAATGGGATTTTCAACAACAGCTGGGGTTACGTCCGTCCCAAACCTCGGACTGCAC  
 GCCACCTATTACAGCCTCAACCGCTTCGGCAGCCAAAGAAGCCCGACGCGTCAGCCGCACT  
 CTACCCATCGTCAACATCGACAGCGGCATGACCTTCGAACGCAATACGCGGATGTTCCGGC  
 GGAGAAGTCTGCAAACCCCTCGAGCCCGCCTGTTCTACAACTATATTCCTGCCAAATCC  
 CAAAACGACCTGCCCAATTTTGATTGCTCGGAAAGCAGCTTCGGCTACGGGCAGCTTTTT  
 30 CGTGAAAACCTCTATTACGGCAACGACAGGATTAACACCGCAAACAGCCTTTCCGCCGCC  
 GTGCAAAGCCGTATTTTGGACGGCGCGACGGGGGCGAGAGCTTTCCGCCGCCGCGCATCGGG  
 CAGAAATTCTACTTCAAAAACGACGCAGTCATGCTTGACGGCAGTGTGCGCAAAAACCG  
 CGCAGCCGTTCCGACTGGGTGGCATTTCGCTCCAGCGGCATCGGCAGCCGCTTCATCTC  
 GACAGCAGCATCCACTACAACCAAAACGACAAACGCGCCGAGAACTACGCCGTGCGGTGCA  
 35 AGCTACCGTCCCGCACAGGGCAAAGTGCTGAACGCCCGCTACAAATACGGGCGCAACGAA  
 AAAATCTACCTGAAGTCCGACGGTTCCTATTTTACGACAACTCAGCCAGCTCGACCTG  
 TCCGCACAATGGCCCCCTGACGCGCAACCTGTGCGCCGTCGTCCGTTACAACTACGGTTTT  
 GAAGCCAAAAACCGATAGAGGTGCTGGCGGGTGCAGGAATACAAAAGCAGTTGCGGGCTGC  
 TGGGGCGCGGGCGTGTACGCCCAACGCTACGTTACCGGCGAAAAACCTACAAAAACGCT  
 GTCTTTTTCTCACTTCAGTTGAAAGACCTCAGCAGTGTGCGCAGAAACCCCGCAGACAGG  
 ATGGATGTGCGCGTTCCCGGCTATATCCCGCCCACTCTCTTCCGCCGGACGCAACAA  
 CGGCCCTGA

#### SEQ ID NO:4

40 *Neisseria meningitidis* BASB054 polypeptide sequence deduced from the polynucleotide sequence of SEQ ID NO:3

45 MARLFSLKPLVLALGFCFGTHCAAADAVAAEETDNPTAGGSVRSVSEPMQAPGLSLGSTC  
 LFCSNESGKPEKTESAVKSGEGPVPENHTRIVADKVEGQSQVKVRAEGGVVERNRTTL  
 NADWADYDQSGDVTVGDRFALQDGTILIRGETLTYNLEQQTGEAHNVRMETEQGRRRLQ  
 SVSRTAEMLGEGHYKLTTETQFNTCSAGDAGWYVKAASVEADREKIGVAKHAAFVFGGVP  
 IFYTPWADFPDGNRKSGLLVPSLSAGSDGVSLSPYFFNLAPNLDAFAPSVIGERGAV  
 FDGQVRYLRPDYAGQSDLTWLPDCKSGRNRYQAKWQHRHDSITLQAGVDFNQVSDSG  
 YYRDFYGNKEIAGNVNLRVWLDYGGRAAGGSLNAGLSVLKYQTLANQSGYKDKPYALM  
 50 PRLSADWRKNTGRAQIGVSAQFTRFSDSRQDGSRLVVYPDIKWDFSNSWGYVRPKLGLH  
 ATYYSLNRFGSQEARRVSRTLPIVNIDSGMTFERNTRMFGGEVLQTLPEPLFYNYIPAKS

QNDLPNFDSSSESSFGYGQLFRENLYYGNDRINTANSLSAVQSRILDGATGAERFRAGIG  
 QKFYFKNDVAMLDGSGVKKPRSRSDWVAFASSGIGSRFILDSSIHYNQNDKRAENYAVGA  
 SYRPAQGVNLNARYKYGRNEKIYLSKSDGSYFYDKLSQLDLAQLWPLTRNLSAVVRYNYGF  
 EAKKPIEVLAGAIEYKSSCGCWGAGVYAQRYVTGENTYKNAVFFSLQLKDLSSVGRNPADR  
 5 MDVAVPGYIPAHSLSAGRNRKP

# SEQ ID NO:5

*Neisseria meningitidis* BASB068 polynucleotide sequence from strain ATCC 13090

10 ATGAAACTCGAAGCAAGCAAGCAAGCAAGCAAGCAGAAGTTTAAAAAATCATTTATTATA  
 AGTCTATTTTTTTCTATTCTTTATACCTCTCCGCTTTTGGCTGTTGATTACGTTTACGAC  
 AAAACCAAGCTCACTGATGATGAAATTACCCGCTTAAAAAACTCCGCGATAGAAATAGT  
 GAATATTGGAAAGAAGAACTTATCACATAAAAAGTAACAACCGAGTTTATCCAAACATT  
 CCCGCATTATCCCTAAACATCCTTTTCGATCCATTGAAAACATCAATAATTCAAAAAGG  
 ATTTCTTTTATGACAAAGAATACACTGAAGATTACCTTGTGGTTTTGCTCAAGGTTTA  
 15 GCGCTTGCAAAAAGAAATGGGGAAACAGAAAAACCAATACGGCAATATTTTAAGGAATGT  
 TTAAACACTGGGAAATATAGTGATGATACTTGCAAATCTCAACAATCTATTCTACAGTA  
 AGAAGTGATATTTTCGCCCTAAATACGAAAATAAAAAATAGCCATATCAACAGCGAAATT  
 TTCGCTGTGCGTAATTATACAAAATTGATGTACTCAGCCCAACATCATTCTATTTGGTCA  
 20 GAGCATCTCTATTCCAATTCAGAATTATCTCTTGACGTTGATAACTCACACGTTATCGGA  
 CAAACGATTGATTGCGGAGCATTAGAATTAACAAATTCTCTATGGGAACCCCGTTGGAAC  
 TCTAATATTGATTATTTAATAACAAAAATGCTGAAATTCGTTTTAACACTAAAAGTGAA  
 AGTTTACTCGTAAAGAAGATTATGCTGGCGGAGCTCGTTTTCGTTTTGCTTACGGTCTA  
 AAAGATAAAGTCCCTGAAACACCAATTTTAACTTTTGAAAAAATATAACTGGCACATCA  
 25 GATATTATTTTTGAGGGAAAAGCGTTGGATAATTTAAACACCTAGACGGGCATCAAATT  
 ATCAAAGTAAATGGCACAGCAGATAAAACAGCATTCCGTCTTTCTGGCAAACACCAAAG  
 GGAATTTATACGCTTTCTTTACAACAACGCCAGAGGGCTTTTTTACCAGAGTGCAAGAA  
 CGCGATGATATTGCGATTATGCACAACAAGCCCAAGCCGCAATACCTTATTCGCCTTG  
 CGTTTAAACGACAAAAACAGCGATATTTTTGACCGCACTTTACCGCGCAAAGGCTTGTGG  
 TTACGTGTGATTGACGGACATTCCAGCCAATGGGTACAAGGCAAAACGGCACCAGTAGAA  
 30 GGAAATCGTAAAGGCATACAACCTTGGTGGCGATGTTTTCTCATTGCAAAATCACAATAT  
 CAACTTTCCGTTGGCTTAATGGGCGGACAAGCAGAACAACGCAGTACTTTCCGCAACCCA  
 GATACAGACAATCTTACAACGGGAAATGTGAAAGGCTTTGGTGAGGCGTTTACGCCACT  
 TGGCATCAGCTTCAGGACAAACAGACAGGTGCGTATGCGGATAGCTGGGTACAATATCAA  
 CGTTTCCGCCACCGTATCAACACTGAAGATGGTACAGAACGTTTACTTCAAAAGGTATT  
 35 ACTGCCTCAATTGAAGCAGGTTACAACGCTTTATTGGCGGAACACGTAATAAAAGGGC  
 AACAGCCTTCGTGTTTACCTACAACCACAGGCGCAATTGACTTATTTGGGGGTAAACGGA  
 AAATTCAGCGATAGCGAAATGCCACGTGAATTTACTTGGCTCTCGCCAATTACAAAGC  
 CGAGTGGGCGTTCAAGCTAAAGCTCAATTTGCTTTCACTAATGGCGTCACTTTCCAACCA  
 TTTGTTTCCGTCAATTCAATCTACCAACAAAACCTTTGGGGGTAGAAATGGACGGAGAA  
 40 CGTCGAGTGATAAACCAACAAACCGCGATTGAAAGCCAATTAGGCGTTGCGGTAAAAATT  
 AAATCTCACTTAACTTTACAAGCAACATTCAACCGCCAAACAGGCAACATCATCAAGCT  
 AAACAAGGCGCATTGAATTTACAGTGGACGTTTAA

# SEQ ID NO:6

45 *Neisseria meningitidis* BASB068 polypeptide sequence deduced from the polynucleotide  
 sequence of SEQ ID NO:5

MKLEASKQASKQKFKKSFIIISLFFSILYTSPLLAVDYVYDKTKLTDDEITRLKKLRDRNS

EYWKEETYHIKSNMRVYPNIPALFPKHPFDPFENINNSKRISFYDKEYTEDYLVGFAQGL  
 GVAKRNGETEKPIRQYFKECLNTGKYSDDTCKSQSIPTVRSDIFALNTKIKNSHINSEI  
 FAVGNYTKLMYSAQHHSIWSEHLYSNSLSLDVDNSHVIGQIDLGALLETNSLWEPRWN  
 SNIDYLITKNAEIRFNTKSESLLVKEDYAGGARFRFAYGLKDKVPETPILTFEKNITGTS  
 5 DIIIFEGKALDNLKHLDDGHQIIKVNGTADKHAFRLSGKHQKGIYTLSQLQRPEGFFTKVQE  
 RDDIAIYAQQAQAANTLFA LRRLNDKNSDIFDRTLPRKGLWLRVIDGHSSQWVQVKTAPE  
 GNRKGIQLGGDVFSLQNHNYQLSVGLMGGQAEQORSTFRNPDTDNLTGTVKGFAGVYAT  
 WHQLQDKQTGAYADSWVQYQRFRRHINTEDGTERFTSKGITASIEAGYNALLAEHVTKKG  
 10 NSLRVYLQPPAQLTYLGVNGKFSSENHVNLLGSRQLQSRVGVQAKAQFAFTNGVTFQP  
 FVSVNSIYQQKPFVEMDGERRVINNKTAIESQLGVAVKIKSHLTLOATFNRQTGKHHQA  
 KQGALNLQWTF

## SEQ ID NO:7

*Neisseria meningitidis* BASB069 polynucleotide sequence from strain ATCC 13090

15 ATGACACTGAAAGCAAGCAAGCAAGCAAGCGGTCGGGTAAATCTATTAACATTATCTGTT  
 TTATCGCTGTTTTGCACGCCATATGTTTGTGGTTCGGATGCGTACGATCCCGTCAAAGAA  
 GCCGAGATTAAAAACAAATTTATTTTAGAAGCGGCGGAAGACAGAAATCCACGTTTGG  
 CGCGGCCCCGTGCAGCATATCTTTTGATTGCTTCGGTATGTTTCAGAGCTCAGCTTGGTTCA  
 AATACTCGTTCTACCAAATCGGCGACGATGCCGATTTTTCATTTTCAGACAAGCCGAAA  
 20 CCCGGCACTTCCCATTTATTTTCCAGCGGTAAACCGATCAAAATTCATCCGAATATGGG  
 TATGACGAAATCAATATCCAAGGTAAACCTACAATAGCGGCATACTCGCCGTCGATAAT  
 ATGCCCGTTGTTAAGAAATATATTACAGATACTTACGGGGATAATTTAAAGGATGCGGTT  
 AAGAAGCAATTACAGGATTTATACAAAACAAGACCCGAAGCTTGGGAAGAAAATAAAAAA  
 CGGACTGAGGAGGCGTATATAGAACAGCTTGGACCAAATTTAGTATACTCAAACAGAAA  
 25 AACCCCGATTTAATTAATAAATTGGTAGAAGATTCGGTACTCACTCCTCATAGTAATACA  
 TCACAGACTAGTCTCAACAACATCTTCAATAAAAAATTACACGTCAAAATCGAAAACAAA  
 TCCCACGTGCGCGGACAGGTGTTGGAAGTACCAAGATGACGCTGAAAGATTCCCTTTGG  
 GAACCGCGCCGCGCATTCCGACATCCATACGCTGGAACTTCCGATAATGCCCGCATCCGC  
 CTGAACACGAAAGATGAAAACTGACCGTCCATAAAGCGTATCAGGGCGGCGCGGATTTC  
 30 CTGTTTCGGCTACGACGTGCGGGAGTTCGGACGAACCCGCCCTGACCTTTGAACAAAACGTC  
 AGCGGAAAATCCGGCGTGGTTTTGGAACGCCGCGGCGGAAAATCTGAAAACGCTCGACGGG  
 CGCAAATGATTGCGGCGGAAAAGGCAGACCCTAATTGCTTTGCGTTTAAACAAAATTAC  
 CGGCAGGGACTGTACGAATTATTGCTCAAGCAATGCGAAGGCGGATTTTGCTTGGGTGTG  
 CAGCGTTTGGCTATCCCTGAGGCGGAAGCGTTTTATATGCCCAACAGGCTTATGCGGCA  
 35 AATACTTTGTTTGGGCTGCGTGCCGCGACAGGGGCGACGACGTGTATGCCGCGGATCCG  
 TCCCGTCAAAAATTGTGGCTGCGCTTCATCGGCGGCCGGTTCGATCAAAATATACGGGGC  
 GGCGCGGCTGCGGACGGGCGGCGCAAAGGCGTGCAAATCGGCGGTGAGGTGTTTGTACGG  
 CAAAATGAAGGCAGTCGGCTGGCAATCGGCGTGATGGGCGGCGAGGCTGGCCAGCAGCA  
 TCAGTCAACGGCAAAGGCGGTGCGGCAGGCAGTTATTTGCATGGTTATGGCGGGGGTGT  
 40 TATGCTGCGTGGCATCAGTTGCGCGATAAACAAACGGGTGCGTATTTGGACGGCTGGTTG  
 CAATACCAACGTTTCAAACACCGCATCAATGATGAAAACCGTGCGGAACGCTACAAAACC  
 AAAGGTTGGACGGCTTCTGTGGAAGGCGGCTACAACGCGCTTGTGGCGGAAGGCGTTGTC  
 GGAAAAGGCAATAATGTGCGGTTTTACCTGCAACCGCAGGCGCAGTTTACCTACTTGGGC  
 GTAAACGGCGGCTTTACCGACAGCGAGGGGACGGCGGTGCGACTGCTCGGCAGCGGTGAG  
 45 TGGCAAAGCCGCGCGGCATTGCGGCAAAAACCGTTTTGCTTTGCGTAACGGTGTCAAT  
 CTTAGCCTTTTGCCGCTTTTAATGTTTTGCACAGGTCAAATCTTTGCGCGTGGAATG  
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50

## SEQ ID NO:8

*Neisseria meningitidis* BASB069 polypeptide sequence deduced from the polynucleotide sequence of SEQ ID NO:7

5 MTLKASKQASGRVNLLTSLVLSLFCTPYVCGSDAYDPVKEAEIKNKFILEAAEDRNSHVW  
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10 SHVAGQVLELTMTLKDLSLWEPRRHSDIHTLETSDNARIRLNTKDEKLTVHKAYQGGADF  
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AGWKGHMSARIGYGKRTDGDKEAALS LKWLF

**Deposited materials**

5 A deposit containing a *Neisseria meningitidis* Serogroup B strain has been deposited with the American Type Culture Collection (herein "ATCC") on June 22, 1997 and assigned deposit number 13090. The deposit was described as *Neisseria meningitidis* (Albrecht and Ghon) and is a freeze-dried, 1.5-2.9 kb insert library constructed from *N. meningitidis* isolate. The deposit is described in Int. Bull. Bacteriol. Nomencl. Taxon. 8: 1-15 (1958).

10 The *Neisseria meningitidis* strain deposit is referred to herein as "the deposited strain" or as "the DNA of the deposited strain."

15 The deposited strain contains the full length BASB047, BASB054, BASB068 and BASB069 genes. The sequence of the polynucleotides contained in the deposited strain, as well as the amino acid sequence of any polypeptide encoded thereby, are controlling in the event of any conflict with any description of sequences herein.

20 The deposit of the deposited strain has been made under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for Purposes of Patent Procedure. The strain will be irrevocably and without restriction or condition released to the public upon the issuance of a patent. The deposited strain is provided merely as convenience to those of skill in the art and is not an admission that a deposit is required for enablement, such as that required under 35 U.S.C. §112.

**INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

<b>A.</b> The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>84</u> , line <u>3-22</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float:right">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution <p align="center"><b>AMERICAN TYPE CULTURE COLLECTION</b></p>	
Address of depositary institution (including postal code and country) <p>10801 UNIVERSITY BLVD, MANASSAS, VIRGINIA 20110-2209, UNITED STATES OF AMERICA</p>	
Date of deposit <p align="center">22 June 1997 (22.06.97)</p>	Accession Number <p align="center">13090</p>
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float:right">This information is continued on an additional sheet <input type="checkbox"/></span>	
<p>In respect of those designations where a European Patent is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European Patent or until the date on which the application has been refused or withdrawn, only by issue of such a sample to an expert nominated by the person requesting the sample.</p>	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

  

<p align="center"><b>For receiving Office use only</b></p> <p><input checked="" type="checkbox"/> This sheet was received with the international application  <div style="display: flex; justify-content: space-around;"> <span><b>19 JAN 2000</b></span> <span>(19. 01. 2000)</span> </div>         Authorized officer  <div style="text-align: center;">   <b>M. Legendre</b> </div> </p>	<p align="center"><b>For International Bureau use only</b></p> <p><input type="checkbox"/> This sheet was received by the International Bureau on:</p> <p>Authorized officer</p>
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## CLAIMS

1. An isolated polypeptide comprising an amino acid sequence which has at least 85%  
5 identity to an amino acid sequence selected from the group consisting of: SEQ ID NO:2,  
SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8.
2. An isolated polypeptide as claimed in claim 1 in which the amino acid sequence has at  
least 95% identity to the amino acid sequence selected from the group consisting of: SEQ  
10 ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8.
3. The polypeptide as claimed in claim 1 comprising the amino acid sequence selected  
from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID  
NO:8.  
15
4. An isolated polypeptide of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 or SEQ ID  
NO:8.
5. An immunogenic fragment of the polypeptide as claimed in any one of claims 1 to 4 in  
20 which the immunogenic activity of said immunogenic fragment is substantially the same  
as that of the polypeptide of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 or SEQ ID  
NO:8.
6. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide  
25 that has at least 85% identity to the amino acid sequence of SEQ ID NO:2,4,6 or 8 over the  
entire length of SEQ ID NO:2,4,6 or 8 respectively; or a nucleotide sequence  
complementary to said isolated polynucleotide.



7. An isolated polynucleotide comprising a nucleotide sequence that has at least 85% identity to a nucleotide sequence encoding a polypeptide of SEQ ID NO:2,4,6 or 8 over the entire coding region; or a nucleotide sequence complementary to said isolated polynucleotide.

5

8. An isolated polynucleotide which comprises a nucleotide sequence which has at least 85% identity to that of SEQ ID NO:1,3,5 or 7 over the entire length of SEQ ID NO:1,3,5 or 7 respectively; or a nucleotide sequence complementary to said isolated polynucleotide.

10 9. The isolated polynucleotide as claimed in any one of claims 6 to 8 in which the identity is at least 95% to SEQ ID NO:1,3,5 or 7.

10. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 or SEQ ID NO:8.

15

11. An isolated polynucleotide comprising the polynucleotide of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7.

20 12. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 or SEQ ID NO:8 obtainable by screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or a fragment thereof.

25 13. An expression vector or a recombinant live microorganism comprising an isolated polynucleotide according to any one of claims 6 to 12.

14. A host cell comprising the expression vector of claim 13 or a subcellular fraction or a membrane of said host cell expressing an isolated polypeptide comprising an amino acid

sequence that has at least 85% identity to the amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8.

- 5 15. A process for producing a polypeptide comprising an amino acid sequence that has at least 85% identity to an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 comprising culturing a host cell of claim 14 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture medium.
- 10 16. A process for expressing a polynucleotide of any one of claims 6 to 12 comprising transforming a host cell with the expression vector comprising at least one of said polynucleotides and culturing said host cell under conditions sufficient for expression of any one of said polynucleotides.
- 15 17. A vaccine composition comprising an effective amount of the polypeptide of any one of claims 1 to 5 and a pharmaceutically acceptable carrier.
- 20 18. A vaccine composition comprising an effective amount of the polynucleotide of any one of claims 6 to 12 and a pharmaceutically effective carrier.
- 25 19. The vaccine composition according to either one of claims 17 or 18 wherein said composition comprises at least one other *Neisseria meningitidis* antigen.
20. An antibody immunospecific for the polypeptide or immunological fragment as claimed in any one of claims 1 to 5.
21. A method of diagnosing a *Neisseria meningitidis* infection, comprising identifying a polypeptide as claimed in any one of claims 1 to 5, or an antibody that is immunospecific

for said polypeptide, present within a biological sample from an animal suspected of having such an infection.

- 5 22. Use of a composition comprising an immunologically effective amount of a polypeptide as claimed in any one of claims 1 to 5 in the preparation of a medicament for use in generating an immune response in an animal.
- 10 23. Use of a composition comprising an immunologically effective amount of a polynucleotide as claimed in any one of claims 6 to 12 in the preparation of a medicament for use in generating an immune response in an animal.
- 15 24. A therapeutic composition useful in treating humans with *Neisseria meningitidis* disease comprising at least one antibody directed against the polypeptide of claims 1 to 5 and a suitable pharmaceutical carrier.

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## SEQUENCE LISTING

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<213> *Neisseria meningitidis*

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<213> *Neisseria meningitidis*

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<213> *Neisseria meningitidis*

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 ttcgctgtcg gtaattatac aaaattgatg tactcagccc aacatcattc tatttggtca 600  
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&lt;210&gt; 6

&lt;211&gt; 671

&lt;212&gt; PRT

<213> *Neisseria meningitidis*

&lt;400&gt; 6

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 20 25 30  
 Leu Ala Val Asp Tyr Val Tyr Asp Lys Thr Lys Leu Thr Asp Asp Glu  
 35 40 45  
 Ile Thr Arg Leu Lys Lys Leu Arg Asp Arg Asn Ser Glu Tyr Trp Lys  
 50 55 60  
 Glu Glu Thr Tyr His Ile Lys Ser Asn Asn Arg Val Tyr Pro Asn Ile  
 65 70 75 80  
 Pro Ala Leu Phe Pro Lys His Pro Phe Asp Pro Phe Glu Asn Ile Asn  
 85 90 95  
 Asn Ser Lys Arg Ile Ser Phe Tyr Asp Lys Glu Tyr Thr Glu Asp Tyr  
 100 105 110  
 Leu Val Gly Phe Ala Gln Gly Leu Gly Val Ala Lys Arg Asn Gly Glu  
 115 120 125  
 Thr Glu Lys Pro Ile Arg Gln Tyr Phe Lys Glu Cys Leu Asn Thr Gly  
 130 135 140  
 Lys Tyr Ser Asp Asp Thr Cys Lys Ser Gln Gln Ser Ile Pro Thr Val  
 145 150 155 160  
 Arg Ser Asp Ile Phe Ala Leu Asn Thr Lys Ile Lys Asn Ser His Ile  
 165 170 175  
 Asn Ser Glu Ile Phe Ala Val Gly Asn Tyr Thr Lys Leu Met Tyr Ser  
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 Ala Gln His His Ser Ile Trp Ser Glu His Leu Tyr Ser Asn Ser Glu

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225	230	235
Ser Asn Ile Asp Tyr Leu Ile Thr Lys Asn Ala Glu Ile Arg Phe Asn		240
245	250	255
Thr Lys Ser Glu Ser Leu Leu Val Lys Glu Asp Tyr Ala Gly Gly Ala		
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Arg Phe Arg Phe Ala Tyr Gly Leu Lys Asp Lys Val Pro Glu Thr Pro		
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Ile Leu Thr Phe Glu Lys Asn Ile Thr Gly Thr Ser Asp Ile Ile Phe		
290	295	300
Glu Gly Lys Ala Leu Asp Asn Leu Lys His Leu Asp Gly His Gln Ile		
305	310	315
Ile Lys Val Asn Gly Thr Ala Asp Lys His Ala Phe Arg Leu Ser Gly		320
325	330	335
Lys His Gln Lys Gly Ile Tyr Thr Leu Ser Leu Gln Gln Arg Pro Glu		
340	345	350
Gly Phe Phe Thr Lys Val Gln Glu Arg Asp Asp Ile Ala Ile Tyr Ala		
355	360	365
Gln Gln Ala Gln Ala Ala Asn Thr Leu Phe Ala Leu Arg Leu Asn Asp		
370	375	380
Lys Asn Ser Asp Ile Phe Asp Arg Thr Leu Pro Arg Lys Gly Leu Trp		
385	390	395
Leu Arg Val Ile Asp Gly His Ser Ser Gln Trp Val Gln Gly Lys Thr		400
405	410	415
Ala Pro Val Glu Gly Asn Arg Lys Gly Ile Gln Leu Gly Gly Asp Val		
420	425	430
Phe Ser Leu Gln Asn His Asn Tyr Gln Leu Ser Val Gly Leu Met Gly		
435	440	445
Gly Gln Ala Glu Gln Arg Ser Thr Phe Arg Asn Pro Asp Thr Asp Asn		
450	455	460
Leu Thr Thr Gly Asn Val Lys Gly Phe Gly Ala Gly Val Tyr Ala Thr		
465	470	475
Trp His Gln Leu Gln Asp Lys Gln Thr Gly Ala Tyr Ala Asp Ser Trp		480
485	490	495
Val Gln Tyr Gln Arg Phe Arg His Arg Ile Asn Thr Glu Asp Gly Thr		
500	505	510
Glu Arg Phe Thr Ser Lys Gly Ile Thr Ala Ser Ile Glu Ala Gly Tyr		
515	520	525
Asn Ala Leu Leu Ala Glu His Val Thr Lys Lys Gly Asn Ser Leu Arg		
530	535	540
Val Tyr Leu Gln Pro Gln Ala Gln Leu Thr Tyr Leu Gly Val Asn Gly		
545	550	555
Lys Phe Ser Asp Ser Glu Asn Ala His Val Asn Leu Leu Gly Ser Arg		560
565	570	575

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 Thr Asn Gly Val Thr Phe Gln Pro Phe Val Ser Val Asn Ser Ile Tyr  
 595 600 605  
 Gln Gln Lys Pro Phe Gly Val Glu Met Asp Gly Glu Arg Arg Val Ile  
 610 615 620  
 Asn Asn Lys Thr Ala Ile Glu Ser Gln Leu Gly Val Ala Val Lys Ile  
 625 630 635 640  
 Lys Ser His Leu Thr Leu Gln Ala Thr Phe Asn Arg Gln Thr Gly Lys  
 645 650 655  
 His His Gln Ala Lys Gln Gly Ala Leu Asn Leu Gln Trp Thr Phe  
 660 665 670

&lt;210&gt; 7

&lt;211&gt; 2076

&lt;212&gt; DNA

<213> *Neisseria meningitidis*

&lt;400&gt; 7

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tggaagcc gcgcggcat tcgggcaaaa acccgttttg ctttgcgtaa cgggtgtcaat 1860
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gacggcgaaa aacagacgct ggcaggcagg acggcgctcg aagggcggtt cggcattgaa 1980
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&lt;210&gt; 8

&lt;211&gt; 691

&lt;212&gt; PRT

<213> *Neisseria meningitidis*

&lt;400&gt; 8

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Thr. Leu. Ser. Val. Leu. Ser. Leu. Phe. Cys. Thr. Pro. Tyr. Val. Cys. Gly. Ser.
20          25          30
Asp. Ala. Tyr. Asp. Pro. Val. Lys. Glu. Ala. Glu. Ile. Lys. Asn. Lys. Phe. Ile.
35          40          45
Leu. Glu. Ala. Ala. Glu. Asp. Arg. Asn. Ser. His. Val. Trp. Arg. Gly. Pro. Cys.
50          55          60
Ser. Ile. Ser. Phe. Asp. Cys. Phe. Gly. Met. Phe. Arg. Ala. Gln. Leu. Gly. Ser.
65          70          75          80
Asn. Thr. Arg. Ser. Thr. Lys. Ile. Gly. Asp. Asp. Ala. Asp. Phe. Ser. Phe. Ser.
85          90          95
Asp. Lys. Pro. Lys. Pro. Gly. Thr. Ser. His. Tyr. Phe. Ser. Ser. Gly. Lys. Thr.
100         105         110
Asp. Gln. Asn. Ser. Ser. Glu. Tyr. Gly. Tyr. Asp. Glu. Ile. Asn. Ile. Gln. Gly.
115         120         125
Lys. Asn. Tyr. Asn. Ser. Gly. Ile. Leu. Ala. Val. Asp. Asn. Met. Pro. Val. Val.
130         135         140
Lys. Lys. Tyr. Ile. Thr. Asp. Thr. Tyr. Gly. Asp. Asn. Leu. Lys. Asp. Ala. Val.
145         150         155         160
Lys. Lys. Gln. Leu. Gln. Asp. Leu. Tyr. Lys. Thr. Arg. Pro. Glu. Ala. Trp. Glu.
165         170         175
Glu. Asn. Lys. Lys. Arg. Thr. Glu. Glu. Ala. Tyr. Ile. Glu. Gln. Leu. Gly. Pro.
180         185         190
Lys. Phe. Ser. Ile. Leu. Lys. Gln. Lys. Asn. Pro. Asp. Leu. Ile. Asn. Lys. Leu.
195         200         205
Val. Glu. Asp. Ser. Val. Leu. Thr. Pro. His. Ser. Asn. Thr. Ser. Gln. Thr. Ser.
210         215         220
Leu. Asn. Asn. Ile. Phe. Asn. Lys. Lys. Leu. His. Val. Lys. Ile. Glu. Asn. Lys.
225         230         235         240
Ser. His. Val. Ala. Gly. Gln. Val. Leu. Glu. Leu. Thr. Lys. Met. Thr. Leu. Lys.
245         250         255
Asp. Ser. Leu. Trp. Glu. Pro. Arg. Arg. His. Ser. Asp. Ile. His. Thr. Leu. Glu.

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Asp Val Arg Glu Ser Asp Glu Pro Ala Leu Thr Phe Glu Gln Asn Val		
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Ser Gly Lys Ser Gly Val Val Leu Glu Arg Arg Pro Glu Asn Leu Lys		
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Thr Leu Asp Gly Arg Lys Leu Ile Ala Ala Glu Lys Ala Asp Pro Asn		
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Ser Phe Ala Phe Lys Gln Asn Tyr Arg Gln Gly Leu Tyr Glu Leu Leu		
355	360	365
Leu Lys Gln Cys Glu Gly Gly Phe Cys Leu Gly Val Gln Arg Leu Ala		
370	375	380
Ile Pro Glu Ala Glu Ala Val Leu Tyr Ala Gln Gln Ala Tyr Ala Ala		
385	390	395
Asn Thr Leu Phe Gly Leu Arg Ala Ala Asp Arg Gly Asp Asp Val Tyr		
405	410	415
Ala Ala Asp Pro Ser Arg Gln Lys Leu Trp Leu Arg Phe Ile Gly Gly		
420	425	430
Arg Ser His Gln Asn Ile Arg Gly Gly Ala Ala Ala Asp Gly Arg Arg		
435	440	445
Lys Gly Val Gln Ile Gly Gly Glu Val Phe Val Arg Gln Asn Glu Gly		
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Ser Arg Leu Ala Ile Gly Val Met Gly Gly Arg Ala Gly Gln His Ala		
465	470	475
Ser Val Asn Gly Lys Gly Gly Ala Ala Gly Ser Tyr Leu His Gly Tyr		
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Gly Gly Gly Val Tyr Ala Ala Trp His Gln Leu Arg Asp Lys Gln Thr		
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Gly Ala Tyr Leu Asp Gly Trp Leu Gln Tyr Gln Arg Phe Lys His Arg		
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Ile Asn Asp Glu Asn Arg Ala Glu Arg Tyr Lys Thr Lys Gly Trp Thr		
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Ala Ser Val Glu Gly Gly Tyr Asn Ala Leu Val Ala Glu Gly Val Val		
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Gly Lys Gly Asn Asn Val Arg Phe Tyr Leu Gln Pro Gln Ala Gln Phe		
565	570	575
Thr Tyr Leu Gly Val Asn Gly Gly Phe Thr Asp Ser Glu Gly Thr Ala		
580	585	590
Val Gly Leu Leu Gly Ser Gly Gln Trp Gln Ser Arg Ala Gly Ile Arg		
595	600	605
Ala Lys Thr Arg Phe Ala Leu Arg Asn Gly Val Asn Leu Gln Pro Phe		
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Ala Ala Phe Asn Val Leu His Arg Ser Lys Ser Phe Gly Val Glu Met		
625	630	635
		640

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Phe Gly Ile Glu Ala Gly Trp Lys Gly His Met Ser Ala Arg Ile Gly  
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Tyr Gly Lys Arg Thr Asp Gly Asp Lys Glu Ala Ala Leu Ser Leu Lys  
675 680 685  
Trp Leu Phe  
690

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